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(54) Title: STABILIZED COMPOSITIONS OF FLUORINATED AMPHIPHILES AS CONTRAST AGENTS FOR ULTRASOUND (57) Abstract Stabilized compositions comprising, in combination with a gas, a fluorinated amphiphilic compound. The compositions are particularly suitable for use in diagnostic applications, including ultrasound. The compositions can take the form of vesicular compositions, such as micelles and liposomes.		

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STABILIZED COMPOSITIONS OF FLUORINATED AMPHIPHILES AS CONTRAST AGENTS FOR ULTRASOUND

Field of the Invention

The present invention relates to novel compositions for ultrasound.

- 5 More particularly, the present invention relates to novel compositions of fluorinated amphiphilic compounds for use as contrast agents for ultrasound.

Background of the Invention

- Ultrasound is a valuable diagnostic imaging technique for studying various areas of the body including, for example, the vasculature, such as tissue
- 10 microvasculature. Ultrasound provides certain advantages relative to other diagnostic techniques. For example, diagnostic techniques involving nuclear medicine and X-rays generally results in exposure of the patient to ionizing electron radiation. Such radiation can cause damage to subcellular material, including deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and proteins. Ultrasound does not involve such
- 15 potentially damaging radiation. In addition, ultrasound is relatively inexpensive as compared, for example, to computed tomography (CT) and magnetic resonance imaging (MRI), which require elaborate and expensive equipment.

Ultrasound involves the exposure of a patient to sound waves.

- Generally, the sound waves dissipate due to absorption by body tissue, penetrate
- 20 through the tissue or reflect off of the tissue. The reflection of sound waves off of tissue, generally referred to as backscatter or reflectivity, forms the basis for developing an ultrasound image. In this connection, sound waves reflect differentially from different body tissues. This differential reflection is due to various factors,

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including, for example, the constituents and the density of the particular tissue being observed. The differentially reflected waves are detected, typically with a transducer that can detect sound waves having a frequency of one megahertz (MHz) to ten MHz. The detected waves can be integrated, quantitated and converted into an image of the tissue being studied.

Ultrasound imaging techniques typically involve the use of contrast agents. Contrast agents are used to improve the quality and usefulness of images which are obtained via ultrasound. Exemplary contrast agents include, for example, suspensions of solid particles, emulsified liquid droplets, and gas-filled bubbles. *See, e.g.,* Hilmann et al., U.S. Patent No. 4,466,442, and published International Patent Applications WO 92/17212 and WO 92/21382.

The quality of images produced from ultrasound has improved significantly. Nevertheless, further improvement is needed, particularly with respect to images involving vasculature in tissues that are perfused with a vascular blood supply. Accordingly, there is a need for improved ultrasound techniques, including improved contrast agents, which are capable of providing medically useful images of the vasculature and vascular-related organs.

The reflection of sound from a liquid-gas interface is extremely efficient. Accordingly, bubbles, including gas-filled bubbles, are useful as contrast agents. The term "bubbles", as used herein, refers to vesicles which are generally characterized by the presence of one or more membranes or walls surrounding an internal void that is filled with a gas or a precursor thereto. Exemplary bubbles include, for example, liposomes, micelles and the like. As discussed more fully hereinafter, the effectiveness of bubbles as contrast agents depends upon various factors, including, for example, the size, elasticity and/or stability of the bubble.

With respect to the effect of bubble size, the signal that is reflected off of a bubble is a function of the radius (r^6) of the bubble (Rayleigh Scatterer). Thus, a bubble having a diameter of 4 micrometer (μm) possesses about 64 times the scattering ability of a bubble having a diameter of 2 μm . Thus, generally speaking, the larger the bubble, the greater the reflected signal.

However, bubble size is limited by the diameter of capillaries through which the bubbles must pass. Contrast agents which comprise bubbles having a

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diameter of greater than 10 μm are generally dangerous since microvessels may be occluded. Accordingly, it is desired that greater than about 99% of the bubbles in a contrast agent have a diameter of less than 10 μm . Mean bubble diameter is important also, and should be greater than 1 μm , with greater than 2 μm being preferred. The volume weighted mean diameter of the bubbles should be about 7 to 10 μm .

Bubble elasticity is also important because highly elastic bubbles can deform, as necessary, to "squeeze" through capillaries. This decreases the likelihood of occlusion. In addition, resonance is more easily induced in bubbles having enhanced elasticity. This can be advantageous in that resonating bubbles typically generate sound emissions at frequencies in the subharmonic regime (based on multiples of 0.5) or in the supra- or ultraharmonic regime (based on multiples of 2). The supraharmonic regime, including second harmonic imaging, is desirable in ultrasound since background noise is substantially eliminated. Elastic bubbles can therefore be used to produce desirable second harmonic images.

The effectiveness of a contrast agent involving bubbles is also dependent on the bubble concentration. Generally, the higher the bubble concentration, the greater the reflectivity of the contrast agent.

Another important characteristic which is related to the effectiveness of bubbles as contrast agents is bubble stability. As used herein, particularly with reference to gas-filled bubbles, "bubble stability" refers to the ability of bubbles to retain gas entrapped therein after exposure to a pressure greater than atmospheric pressure. To be effective as contrast agents, bubbles generally need to retain an amount of the entrapped gas *in vivo*. It is also highly desirable that, after release of the pressure, the bubbles return to their original size. This is referred to generally as "bubble resilience."

Bubbles which lack desirable stability provide poor contrast agents. If, for example, bubbles release the gas entrapped therein *in vivo*, reflectivity is diminished. Similarly, the size of bubbles which possess poor resilience will be decreased *in vivo*, also resulting in diminished reflectivity.

The stability of bubbles disclosed in the prior art is generally inadequate for use as contrast agents. For example, the prior art discloses bubbles,

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including gas-filled liposomes, which comprise lipoidal walls or membranes. *See, e.g.,* Ryan et al., U.S. Patent Nos. 4,900,540 and 4,544,545; Tickner et al., U.S. Patent No. 4,276,885; Klaveness et al., WO 93/13809 and Schneider et al., EPO 0 554 213 and WO 91/15244. The stability of the bubbles disclosed in these references is poor in that as the solutions in which the bubbles are suspended become diluted, for example, *in vivo*, the walls or membranes of the bubbles are thinned. This results in a greater likelihood of rupture of the bubbles.

Various attempts have been made to improve bubble stability. Such attempts have included, for example, the preparation of bubbles in which the membranes or walls thereof are apparently strengthened via crosslinking. *See, e.g.,* 10 Giddey et al., U.S. Patent No. 5,310,540 and Klaveness et al., WO 92/17212, in which there are disclosed bubbles which comprise proteins crosslinked with crosslinking agents.

Prior art techniques for stabilizing bubbles, including the use of crosslinked materials, suffer from various drawbacks. For example, the crosslinked materials described, for example, in Giddey et al., U.S. Patent No. 5,310,540 and Klaveness et al., WO 92/17212, lack biocompatibility or possess unknown metabolic fates. Added costs are also incurred with the use of additional materials and process steps necessary for crosslinking. In addition, crosslinking can impart rigidity to the membranes or walls of the bubbles. This results in bubbles having reduced elasticity and, therefore, a decreased ability to deform and pass through capillaries. Thus, there is a greater likelihood of occlusion of vessels with prior art contrast agents that are stabilized via crosslinking.

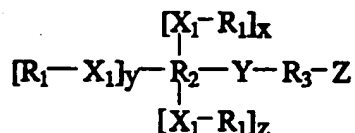
Accordingly, new and/or better stabilized contrast agents and methods for providing same are needed. The present invention is directed to this, as well as other, important ends.

Summary of the Invention

The present invention is directed, in part, to stabilized compositions of a gas and a sulfonated or phosphorylated fluorinated amphiphilic compound. Specifically, in one aspect, the present invention relates to a stabilized composition

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comprising, in combination with a gas, a compound of the formula



wherein:

(I)

each of x, y and z is independently 0 or 1;

each X_1 is independently -O-, -S-, -SO-, -SO₂-, -NR₄-, -C(=X₂)-,

5 -C(=X₂)-O-, -O-C(=X₂)-, -C(=X₂)-NR₄- or -NR₄-C(=X₂)-;

X_2 is O or S;

Y is a direct bond or -X₃-M(=O)(OR₅)_q-O-, where q is 1 or 2;

X_3 is a direct bond or -O-;

M is P or S;

10 Z is hydrogen, the residue of a hydrophilic polymer, a saccharide residue or -N(R₆)_r, where r is 2 or 3;

each R₁ is independently alkyl of 1 to about 30 carbons or fluorinated alkyl of 1 to about 30 carbons;

15 R₂ is a direct bond or an alkylene linking group of 1 to about 10 carbons;

R₃ is a direct bond or an alkylene diradical of 1 to about 10 carbons;

each of R₄ and R₅ is independently hydrogen or alkyl of 1 to about 8 carbons; and

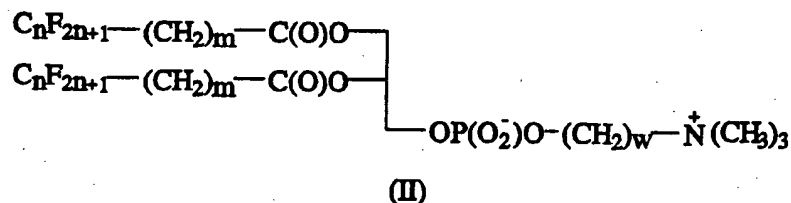
20 each R₆ is independently hydrogen, alkyl of 1 to about 8 carbons or a residue of a hydrophilic polymer;

provided that at least one of x, y and z is 1, at least one of R₁ is fluorinated alkyl of 1 to about 30 carbons and when R₂ is a direct bond, at least two of x, y and z are 0. In certain embodiments, the composition may further comprise, as desired, a gaseous

25 precursor. Also, if desired, the composition may further comprise a bioactive agent.

Another aspect of the invention relates to compositions comprising, in combination with a gas, a compound of the formula

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wherein:

m is 0 to about 18;

n is 1 to about 12; and

w is 1 to about 8.

5 Yet another aspect of the invention relates to a method for the preparation of a stabilized composition of a fluorinated amphiphilic compound and a gas. The method comprises agitating an aqueous mixture of a fluorinated amphiphilic compound in the presence of a gas.

10 Still another aspect of the invention relates to a method of providing an image of an internal region of a patient. The method comprises administering to the patient a contrast medium comprising a stabilized composition comprising a gas and a fluorinated amphiphilic compound. The method further comprises scanning the patient using ultrasound to obtain visible images of the region.

15 Another aspect of the invention relates also to a method of providing an image of an internal region of a patient. This method comprises administering to the patient a vesicular composition comprising, in an aqueous carrier, vesicles comprising a fluorinated amphiphilic compound and a gas or gaseous precursor. The method also involves scanning the patient using ultrasound to obtain a visible image of any diseased tissue in the patient.

20 Yet another aspect of the invention relates to a method for diagnosing the presence of diseased tissue in a patient. The method involves administering to the patient a contrast medium comprising a stabilized composition comprising a gas and a

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fluorinated amphiphilic compound. The method further involves scanning the patient using ultrasound to obtain visible images of any diseased tissue in the patient.

Still another aspect of the invention relates to a method for the therapeutic delivery *in vivo* of a bioactive agent. The method comprises
5 administering to a patient a therapeutically effective amount of a formulation which comprises, in combination with a bioactive agent, a stabilized composition of a fluorinated amphiphilic compound and a gas.

These and other aspects of the invention will become more apparent from the present specification and claims.

10 Detailed Description of the Invention

As employed above and throughout the disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings.

"Amphiphile" or "amphiphilic compound" refers to a synthetic or naturally-occurring compound having a water-soluble, hydrophilic portion and a
15 water-insoluble, hydrophobic portion. Preferred amphiphilic compounds are characterized by a polar head group, for example, a phosphatidylcholine group, and one or more nonpolar, aliphatic chains, for example, palmitoyl groups. "Fluorinated amphiphile" refers to an amphiphilic compound in which at least one hydrogen atom of the amphiphilic compound is replaced with a fluorine atom. In preferred form, the
20 fluorinated amphiphilic compounds are polyfluorinated. "Polyfluorinated", as used herein, refers to amphiphilic compounds which contain two or more fluorine atoms. In certain preferred embodiments of the present invention, the amphiphilic compounds comprise a lipid. "Lipid", as used herein, refers to materials, including fats and fat-derived materials, which are relatively insoluble in water but relatively soluble in
25 organic solvents, such as benzene, chloroform, acetone and ether. Lipids include, for example, fatty acids, fatty acid esters, neutral fats, phosphatides (phospholipids), glycolipids, fatty alcohols, sterols, waxes, terpenes and steroids. As discussed in detail below, certain preferred lipid compounds are phosphorylated and contain phosphate groups, for example, PO₄ groups, and certain other preferred lipid
30 compounds are sulfated and contain sulfate groups, for example, SO₄ groups.

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"Amphipathy" refers to the simultaneous attraction and repulsion in a single molecule or ion containing one or more groups having an affinity for the phase or medium in which they are dissolved; emulsified and/or suspended, together with one or more groups that tend to be expelled from the involved phase or medium.

5 "Stabilized" refers to compositions which have been formulated as a mixture of finely divided colloidal particles floating in a liquid with minimal aggregation. As discussed in detail below, certain preferred embodiments of the present invention involve compositions of stabilized vesicles. In this context, the term "stabilized" refers to vesicles which are substantially resistant to degradation that
10 is caused, for example, by the loss of structural or compositional integrity in the walls of the vesicles and/or by the loss of any significant portion of a gas or gaseous precursor encapsulated within the vesicle.

"Amphiphilic composition" refers to a composition which comprises an amphiphilic compound. Exemplary amphiphilic compositions include suspensions,
15 emulsions and vesicular compositions.

"Suspension" refers to a mixture, dispersion or emulsion of finely divided colloidal particles floating in a liquid. The particles may be solid, liquid or gaseous.

"Emulsion" refers to a mixture of two or more liquids and is generally
20 in the form of a colloidal mixture.

"Vesicle" refers to a spherical entity which is characterized by the presence of an internal void. Preferred vesicles are formulated from amphiphilic compounds, including lipids. In any given vesicle, the amphiphilic compounds may be in the form of a monolayer or bilayer, and the mono- or bilayer amphiphiles may
25 be used to form one or more mono- or bilayers. In the case of more than one mono- or bilayer, the mono- or bilayers are generally concentric. The vesicles described herein include such entities commonly referred to as liposomes, micelles, bubbles, microbubbles, microspheres and the like. Thus, the amphiphilic compounds may be used to form a unilamellar vesicle (comprised of one monolayer or bilayer), an
30 oligolamellar vesicle (comprised of about two or about three monolayers or bilayers) or a multilamellar vesicle (comprised of more than about three monolayers or bilayers). The internal void of the vesicles may be filled with a liquid, including, for

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example, an aqueous liquid, a gas, a gaseous precursor, and/or a solid or solute material, including, for example, a bioactive agent, as desired.

"Liposome" refers to a generally spherical cluster or aggregate of amphiphilic compounds, including lipid compounds, typically in the form of one or
5 more concentric layers, for example, bilayers. They may also be referred to herein as vesicles or amphiphilic vesicles.

"Gas filled vesicles" refers to vesicles in which there is encapsulated a gas. "Gaseous precursor filled vesicles" refers to vesicles in which there is encapsulated a gaseous precursor. The vesicles may be minimally, partially or
10 substantially completely filled with the gas and/or gaseous precursor. In preferred embodiments, the vesicles are substantially completely filled with the gas and/or gaseous precursor.

"Vesicular composition" refers to a composition of vesicles that is prepared from amphiphilic compounds.

15 "Amphiphilic formulation" refers to a composition which comprises an amphiphilic compound and a bioactive agent.

"Vesicle formulation" refers to a composition which comprises vesicles and a bioactive agent.

"Patient" refers to animals, including mammals, preferably humans.

20 "Biocompatible" refers to materials which are generally not injurious to biological functions and which will not result in any degree of unacceptable toxicity, including allergenic responses and disease states.

"Bioactive agent" refers to a substance which is used in connection with an application that is therapeutic or diagnostic in nature, such as in methods for
25 diagnosing the presence or absence of a disease in a patient and/or in methods for the treatment of disease in a patient. As used herein, "bioactive agent" refers also to substances which are capable of exerting a biological effect *in vitro* and/or *in vivo*. The bioactive agents may be neutral or positively or negatively charged. Examples of suitable bioactive agents include diagnostic agents, pharmaceuticals, drugs, synthetic
30 organic molecules, proteins, peptides, vitamins, steroids and genetic material, including nucleosides, nucleotides and polynucleotides.

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"Diagnostic agent" refers to any agent which is used in connection with methods for diagnosing the presence or absence of a disease in a patient. Exemplary diagnostic agents include, for example, contrast agents for use in connection with ultrasound, magnetic resonance imaging or computed tomography of a patient.

5 "Genetic material" refers generally to nucleotides and polynucleotides, including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The genetic material may be made by synthetic chemical methodology known to one of ordinary skill in the art, or by the use of recombinant technology, or by a combination of the two. The DNA and RNA may optionally comprise unnatural nucleotides and may be
10 single or double stranded. "Genetic material" refers also to sense and anti-sense DNA and RNA, that is, a nucleotide sequence which is complementary to a specific sequence of nucleotides in DNA and/or RNA.

"Pharmaceutical" or "drug" refers to any therapeutic or prophylactic agent which is used in the treatment, including the prevention, diagnosis, alleviation,
15 or cure, of a malady, affliction, disease or injury in a patient. Therapeutically useful peptides, polypeptides and polynucleotides are included within the meaning of the term pharmaceutical or drug.

"Polymer", as used herein, refers to molecules formed from the chemical union of two or more repeating units. Accordingly, included within the
20 term "polymer" are, for example, dimers, trimers and oligomers.

"Aliphatic" refers to one of the major groups of organic compounds, characterized by straight- or branched-chain arrangement of the constituent carbon atoms. Aliphatic hydrocarbons comprise three subgroups: (1) paraffins, including the alkanes; (2) olefins, including the alkenes, which contain carbon-carbon double bonds;
25 and (3) acetylenes, including the alkynes, which contain carbon-carbon triple bonds.

"Alkyl" refers to an aliphatic hydrocarbon group which may be straight or branched having 1 to about 30 carbon atoms. "Lower alkyl" refers to an alkyl group having 1 to about 8 carbon atoms. "Higher alkyl" refers to an alkyl group having about 10 to about 30 carbon atoms. The alkyl group may be optionally
30 substituted with one or more alkyl group substituents which may be the same or different, where "alkyl group substituent" includes, for example, halo, aryl, hydroxy, alkoxy, aryloxy, alkylthio, arylthio, carboxy alkoxycarbonyl, oxo and

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cycloalkyl. There may be optionally inserted along the alkyl group one or more oxygen, sulphur or substituted nitrogen atoms, wherein the nitrogen substituent is lower alkyl. The alkyl group may be linear or branched. "Branched" refers to an alkyl group in which a lower alkyl group, such as methyl, ethyl or propyl, is attached to a linear alkyl chain. The alkyl group can include one or more points of unsaturation including, for example, carbon-carbon double bonds and carbon-carbon triple bonds. Exemplary alkyl groups include, for example, methyl, ethyl, i-propyl, n-butyl, t-butyl, n-pentyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl and hexadecyl. Preferred alkyl groups include the lower alkyl groups having 1 to about 4 carbon atoms and the higher alkyl groups having about 10 to about 16 carbon atoms. Exemplary alkyl groups which contain alkyl group substituents include hydroxylated alkyl groups, such as alkyl groups derived from glycerol, including, for example, 2,3-dihydroxyprop-1-yl.

"Alkylene", when used in conjunction with the term "diradical", refers to a bivalent aliphatic hydrocarbon group having from 1 to about 30 carbon atoms. When used in conjunction with the term "linking group", "alkylene" refers to a bi- or trivalent aliphatic hydrocarbon group having from 1 to about 30 carbons. The alkylene group may be straight, branched or cyclic. The alkylene group may be also optionally unsaturated and/or substituted with one or more "alkyl group substituents."

There may be optionally inserted along the alkylene group one or more oxygen, sulphur or substituted or unsubstituted nitrogen atoms, wherein the nitrogen substituent is alkyl as previously described. Exemplary alkylene groups include methylene ($-\text{CH}_2-$), ethylene ($-\text{CH}_2\text{CH}_2-$), propylene ($-(\text{CH}_2)_3-$), prop-1,2,3-triyl ($-\text{CH}_2-\text{CH}(-)-\text{CH}_2-$), cyclohexylene ($-\text{C}_6\text{H}_{10}-$), $-\text{CH}=\text{CH}-\text{CH}=\text{CH}-$, $-\text{CH}=\text{CH}-\text{CH}_2-$, $-(\text{CF}_2)_n(\text{CH}_2)_m-$, wherein n is an integer from about 1 to about 22 and m is an integer from 0 to about 22, $-(\text{CH}_2)_n-\text{N}(\text{R})-(\text{CH}_2)_m-$, wherein each of m and n is independently an integer from 0 to about 30 and R is hydrogen or alkyl, methylenedioxy ($-\text{O}-\text{CH}_2-\text{O}-$) and ethylenedioxy ($-\text{O}-(\text{CH}_2)_2-\text{O}-$). It is preferred that the alkylene group has about 2 to about 3 carbon atoms.

The present invention is directed, in part, to stabilized compositions which are useful, for example, as contrast agents for diagnostic and/or therapeutic ultrasound. The compositions comprise, in combination with a gas and preferably in

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an aqueous carrier, a fluorinated amphiphilic compound. The fluorinated amphiphilic compounds, which are described in detail below, impart highly desirable properties to the compositions of the present invention. For example, it has been surprisingly and unexpectedly found that the fluorinated amphiphilic compounds are capable of

5 stabilizing the present compositions, including preferred compositions which comprise vesicles. It has been found also that the present fluorinated amphiphilic compounds are capable of promoting the formation of vesicles, as well as improving the stability of the formed vesicles. In embodiments in which the vesicles comprise gas-filled and/or gaseous precursor-filled vesicles, the fluorinated amphiphilic compounds

10 enable the vesicles to substantially retain the gas and/or gaseous precursor with minimal loss or leakage. This is surprising and unexpected and generally renders unnecessary the use of additional stabilizing materials, including, for example, surfactants, and stabilizing techniques, including, for example, crosslinking of the materials in the walls of the vesicles. As discussed above, such techniques are

15 generally necessary in connection with contrast agents of the prior art. Moreover, the present fluorinated amphiphilic compounds are generally biocompatible and can be obtained with minimal effort and at minimal expense. Accordingly, the present invention is directed to simple and efficient methods for providing stabilized compositions, including vesicular compositions, for use as ultrasound contrast agents.

20 A variety of fluorinated amphiphilic compounds can be employed in the present compositions. Preferred fluorinated amphiphilic compounds are those which, when combined with a gas, tend to form stabilized compositions and/or vesicles. Preferred also are fluorinated amphiphilic compounds which are capable of stabilizing the vesicles, once formed.

25 In preferred embodiments, the fluorinated amphiphilic compounds are based on amphiphilic compounds, including lipids, and especially phospholipids, which comprise a polar head group including, for example, a phosphorylated head group, such as a phosphatidylcholine group, or a sulfated head group, and at least one nonpolar aliphatic chain, such as a palmitoyl group. In such embodiments, the

30 fluorine atoms are preferably substituted on the nonpolar aliphatic chain portions of the involved amphiphilic compounds. As noted above, among the preferred amphiphilic compounds are phosphorylated and/or sulfated lipid compounds. It is

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contemplated that the term "phosphorylate", as used herein, encompasses phosphate groups with various valences, including, for example, PO_3 and PO_4 groups. Similarly, it is contemplated that the term "sulfated", as used herein, encompasses sulfate groups with various valences, including, for example, SO_3 and SO_4 . It is contemplated that

5 in these preferred lipid compounds, the phosphate group and/or the sulfate group are preferably located within the backbone portions of the lipid compounds. Thus, generally speaking, the phosphate and/or sulfate groups in the preferred phosphorylated and/or sulfated lipid compounds are desirably spaced from the end-
portions of the compounds with, for example, alkyl groups, and as such, are referred

10 to herein as "internal phosphate (and/or sulfate) groups". These preferred fluorinated amphiphilic compounds can be contrasted with prior art fluorinated compounds, including, for example, the class of compounds which are commercially available as ZONYL™ fluorosurfactants (DuPont Chemical Corp., Wilmington DE), including the ZONYL™ phosphate salts and the ZONYL™ sulfate salts, which have terminal

15 phosphate or sulfate groups. Representatives of these salts are disclosed, for example, in U.S. Patent No. 5,276,146, wherein the ZONYL™ phosphate salt has the formula $[\text{F}(\text{CF}_2\text{CF}_2)_{3-8}\text{CH}_2\text{CH}_2\text{O}]_{1,2}\text{P}(\text{O})(\text{O}^-\text{NH}_4^+)_{2,1}$ and the ZONYL™ sulfate salt has the formula $\text{F}(\text{CF}_2\text{CF}_2)_{3-8}\text{CH}_2\text{CH}_2\text{SCH}_2\text{CH}_2\text{N}^+(\text{CH}_3)_3 \text{OSO}_2\text{OCH}_3$. In contrast to the preferred phosphorylated and sulfated lipid compounds involved in the present

20 invention, the ZONYL™ phosphate and sulfate salts, as depicted above, include phosphate and sulfate moieties in the terminal portions of the disclosed compounds.

In certain preferred embodiments, the fluorinated amphiphilic compounds are polyfluorinated. As noted above, this means that the amphiphilic compounds are preferably substituted with at least two or more fluorine atoms. In

25 even more preferred embodiments, the fluorinated amphiphilic compounds are perfluorinated.

As noted above, preferred amphiphilic compounds on which the fluorinated amphiphiles are based, generally comprise at least one nonpolar aliphatic chain. In certain preferred embodiments, the amphiphilic compounds comprise, at

30 most, one nonpolar aliphatic chain. Such compounds are referred to herein as "monochain compounds." In certain other preferred embodiments, the amphiphilic compounds comprise more than one, and preferably at least two or three, nonpolar

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aliphatic chains. Such compounds are referred to herein as "polychain compounds." Whether monochain or polychain, it is preferred that the fluorine atoms are substituted on the chain portion(s) of the amphiphilic compounds. The extent of the fluorination of the chain(s), and the location of the fluorine atoms on the chain(s), can be selected, as desired, in the fluorinated amphiphilic compounds. For example, only a certain portion of the nonpolar chain group, such as the terminal methyl group (CH_3 -) or an internal methylene group ($-\text{CH}_2-$), can be fluorinated, with the remainder of the nonpolar chain group being, for example, unsubstituted aliphatic, such as unsubstituted alkyl. Alternatively, it is contemplated that a substantial majority of the nonpolar chain group can be fluorinated. The nonpolar chain group can include substantially equivalent portions which are fluorinated and nonfluorinated, as determined, for example, by the number of carbon atoms in the fluorinated and nonfluorinated portions. In preferred form, the nonpolar chain group contains both a nonfluorinated portion and a fluorinated portion.

With particular reference to polychain compounds, it is contemplated that all of the chains can be fluorinated, or only certain of the chains can be fluorinated. In the latter case, the nonfluorinated chain(s) can be, for example, unsubstituted aliphatic, including unsubstituted alkyl. Such compounds are referred to herein as "asymmetric amphiphilic compounds", since both fluorinated and nonfluorinated chains are present in the same molecule.

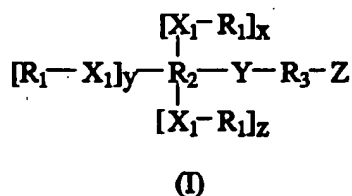
In connection with the preferred embodiments of the present invention involving mono- and polychain amphiphilic compounds, the nonpolar aliphatic chains can be referred to as having a proximal end portion and a distal end portion. The proximal end portion corresponds to that portion of the aliphatic chain which is proximate the polar head group. Thus, in the case of phospholipids, including, for example, phosphatidylcholines, such as mono- and dipalmitoylphosphatidylcholines, the proximal end portions of the palmitoyl moieties correspond to the portions of the chains which are located near the carbonyloxy ($-\text{C}(=\text{O})-\text{O}-$) groups and the glycerolphosphocholine moiety. Similarly, the distal end portions correspond to the portions of the chains which are located near the terminal methyl groups of the alkyl chains of the palmitoyl moieties.

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In preferred embodiments, at least the distal end portion of the nonpolar chain(s) of the mono- and polychain amphiphilic compounds is fluorinated, with polyfluorinated distal ends being more preferred. Even more preferably, the distal end portions of the nonpolar chains are perfluorinated. Also in preferred

5 embodiments, the proximal end portion of the nonpolar chain portion(s) are unsubstituted aliphatic, with unsubstituted alkyl being more preferred. Thus, particularly preferred embodiments of the invention involve fluorinated amphiphilic compounds in which one or more nonpolar aliphatic chains contain both a perfluorinated distal end portion and an unsubstituted proximal end portion.

10 In certain particularly preferred embodiments, the fluorinated amphiphilic compound has the formula



wherein:

- each of x, y and z is independently 0 or 1;
- each X_1 is independently -O-, -S-, -SO-, -SO₂-, -NR₄-, -C(=X₂)-,
- 15 -C(=X₂)-O-, -O-C(=X₂)-, -C(=X₂)-NR₄- or -NR₄-C(=X₂)-;
- X_2 is O or S;
- Y is a direct bond or -X₃-M(=O)(OR₅)_q-O-, where q is 1 or 2;
- X_3 is a direct bond or -O-;
- M is P or S;
- 20 Z is hydrogen, the residue of a hydrophilic polymer, a saccharide residue or -N(R₆)_r, where r is 2 or 3;
- each R₁ is independently alkyl of 1 to about 30 carbons or fluorinated alkyl of 1 to about 30 carbons;
- R₂ is a direct bond or an alkylene linking group of 1 to about 10
- 25 carbons;

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R_3 is a direct bond or an alkylene diradical of 1 to about 10 carbons;

each of R_4 and R_5 is independently hydrogen or alkyl of 1 to about 8 carbons; and

5 each R_6 is independently hydrogen, alkyl of 1 to about 8 carbons or a residue of a hydrophilic polymer;

provided that at least one of x , y and z is 1, at least one of R_1 is fluorinated alkyl of 1 to about 30 carbons, and when R_2 is a direct bond, two of x , y and z are each 0.

In the above formula (I), each of x , y and z is independently 0 or 1, provided that at least one of x , y and z is 1. In certain preferred embodiments, two of x , y and z are each 0. In certain other preferred embodiments, one of x , y and z is 0 or 1 and the other two of x , y and z are each 1, with one of x , y and z being 0 and the other two of x , y and z being 1 being more preferred. In certain other preferred embodiments, each of x , y and z is 1.

15 Each X_1 in formula (I) above is independently -O-, -S-, -SO-, -SO₂-, -NR₄-, -C(=X₂)-, -C(=X₂)-O-, -O-C(=X₂)-, -C(=X₂)-NR₄- or -NR₄-C(=X₂)-. Preferably, each X_1 is independently -O-, -S-, -C(=X₂)-, -C(=X₂)-O-, -O-C(=X₂)-, -C(=X₂)-NR₄- or -NR₄-C(=X₂)-. More preferably, each X_1 is independently -C(=X₂)-O- or -O-C(=X₂)-. Even more preferably, each X_1 is -C(=X₂)-O-.

20 In the above formula (I), each X_2 is O or S. Preferably, each X_2 is O.

In formula (I) above, Y is a direct bond or -X₃-M(=O)(OR₅)_q-O-, where q is 1 or 2. Preferably, Y is -X₃-M(=O)(OR₅)_q-O-.

M in the above definition of Y is P or S. Preferably, M is P.

25 X_3 in the above definition of Y is a direct bond or -O-. Preferably, X_3 is a direct bond.

In formula (I) above, Z is hydrogen, the residue of a hydrophilic polymer, a saccharide residue or -N(R₆)_r, where r is 2 or 3. In preferred embodiments, Z is -N(R₆)_r.

30 Each R_1 in formula (I) is independently alkyl of 1 to about 30 carbons or fluorinated alkyl of 1 to about 30 carbons, provided that at least one of R_1 is fluorinated alkyl of 1 to about 30 carbons. Thus, when only one of x , y and z is 1, R_1 is necessarily fluorinated alkyl of 1 to about 30 carbons. In certain preferred

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embodiments, where one or none of x, y and z is 0, and preferably where one of x, y and z is 0 and the other two of x, y and z are each 1, at least one of R_1 is alkyl of 1 to about 30 carbons and at least one of R_1 is fluorinated alkyl of 1 to about 30 carbons. In other preferred embodiments, each R_1 is independently fluorinated alkyl of 1 to about 30 carbons. When fluorinated alkyl of 1 to about 30 carbons, R_1 is preferably polyfluorinated alkyl of 1 to about 30 carbons, with perfluorinated alkyl of 1 to about 30 carbons being more preferred. When perfluorinated alkyl of 1 to about 30 carbons, R_1 is preferably $C_nF_{2n+1}-(CH_2)_m-$, where n is 1 to about 12 and m is 0 to about 18. In these latter embodiments, n is preferably about 2 to about 10 and m is preferably about 2 to about 14. More preferably, n is about 4 to about 8 and m is about 4 to about 10.

In formula (I) above, R_2 is a direct bond or an alkylene linking group of 1 to about 10 carbons, provided that when R_2 is a direct bond, two of x, y and z are each 0. Preferably, R_2 is a direct bond or an alkylene linking group of 1 to about 4 carbons. More preferably, R_2 is an alkylene linking group of about 3 carbons. Even more preferably, R_2 is $-CH_2-CH(-)-CH_2-$.

R_3 in formula (I) above is a direct bond or an alkylene diradical of 1 to about 10 carbons. Preferably, R_3 is a direct bond or an alkylene diradical of 1 to about 4 carbons. More preferably, R_3 is an alkylene diradical of about 2 carbons. Even more preferably, R_3 is $-CH_2CH_2-$.

In formula (I) above, each of R_4 and R_5 is independently hydrogen or alkyl of 1 to about 8 carbons. Preferably, each of R_4 and R_5 is independently hydrogen or alkyl of 1 to about 4 carbons. More preferably, each of R_4 and R_5 is hydrogen.

R_6 in formula (I) above is hydrogen, alkyl of 1 to about 8 carbons or a residue of a hydrophilic polymer. Preferably, R_6 is hydrogen or alkyl of 1 to about 4 carbons. More preferably, R_6 is hydrogen or methyl, with methyl being even more preferred.

In formula (I) above, it is intended that when any symbol appears more than once in a particular formula or substituent, its meaning in each instance is independent of the other. This independence of meaning is subject to any of the stated provisos.

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Also in formula (I), it is intended that when each of two or more adjacent symbols is defined as being "a direct bond" to provide multiple, adjacent direct bonds, the multiple and adjacent direct bonds devolve into a single direct bond.

As noted above, Z and R₆ in the definition of Z in formula (I), can be,
5 *inter alia*, the residue of a hydrophilic polymer. Exemplary polymers from which Z and/or R₆ can be derived include polymers in which the repeating units contain one or more hydroxy groups (polyhydroxy polymers), including, for example, poly(vinyl alcohol); polymers in which the repeating units contain one or more amino groups (polyamine polymers), including, for example, peptides, polypeptides, proteins and
10 lipoproteins, such as albumin and natural lipoproteins; polymers in which the repeating units contain one or more carboxy groups (polycarboxy polymers), including, for example, carboxymethylcellulose, alginic acid and salts thereof, such as sodium and calcium alginate, glycosaminoglycans and salts thereof, including salts of hyaluronic acid, phosphorylated and sulfonated derivatives of carbohydrates, genetic
15 material, such as interleukin-2 and interferon, and phosphorothioate oligomers; and polymers in which the repeating units contain one or more saccharide moieties (polysaccharide polymers), including, for example, carbohydrates. The molecular weight of the polymers from which Z and/or R₆ are derived may vary, and is generally about 50 to about 5,000,000, with polymers having a molecular weight of
20 about 100 to about 50,000 being preferred. More preferred polymers have a molecular weight of about 150 to about 10,000, with molecular weights of 200 to about 8,000 being even more preferred.

Preferred polymers from which Z and/or R₆ are derived include, for example, poly(ethyleneglycol) (PEG), poly(vinylpyrrolidone), polyoxomers,
25 polysorbate and poly(vinyl alcohol), with PEG polymers being particularly preferred. Preferred among the PEG polymers are PEG polymers having a molecular weight of from about 100 to about 10,000. More preferably, the PEG polymers have a molecular weight of from about 200 to about 8,000, with PEG 2,000, PEG 5,000 and PEG 8,000, which have molecular weights of 2,000, 5,000 and 8,000, respectively,
30 being even more preferred.

Other suitable hydrophilic polymers, in addition to those exemplified above, will be readily apparent to one skilled in the art based on the present

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disclosure. Generally, polymers from which Z and/or R₆ are derived include polymers that can be incorporated in the fluorinated amphiphilic compounds via alkylation or acylation reactions.

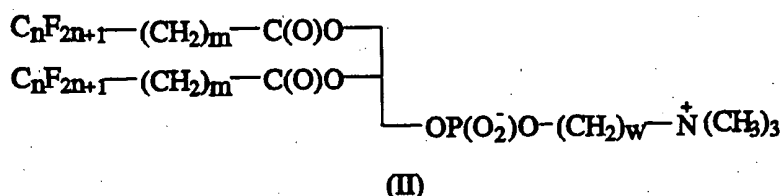
As with the various polymers exemplified above, it is contemplated that the polymeric residues can contain functional groups in addition, for example, to those typically involved in linking the polymeric residues to the fluorinated amphiphilic compounds. Such functionalities include, for example, carboxyl, amine, hydroxy and thiol groups. These functional groups on the polymeric residues can be further reacted, if desired, with materials which are generally reactive with such functional groups and which can assist in targeting specific tissues in the body including, for example, diseased tissue. Exemplary materials which can be reacted with the additional functional groups include, for example, proteins, including antibodies, carbohydrates, peptides, glycopeptides, glycolipids, lectins and nucleosides.

In addition to residues of hydrophilic polymers, Z in formula (I) can also be a saccharide residue. Exemplary saccharides from which Z can be derived include, for example, monosaccharides or sugar alcohols, such as erythrose, threose, ribose, arabinose, xylose, lyxose, fructose, sorbitol, mannitol and sedoheptulose, with preferred monosaccharides being fructose, mannose, xylose, arabinose, mannitol and sorbitol; and disaccharides, such as lactose, sucrose, maltose and cellobiose. Other saccharides include, for example, inositol and ganglioside head groups.

Other suitable saccharides, in addition to those exemplified above, will be readily apparent to one skilled in the art based on the present disclosure. Generally, saccharides from which Z is derived include saccharides that can be incorporated in the fluorinated amphiphilic compounds via alkylation or acylation reactions.

A particularly preferred class of fluorinated amphiphilic compounds of the present invention, which are within the scope of the compound of formula (I), is represented by the formula

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wherein:

m is 0 to about 18;

n is 1 to about 12; and

w is 1 to about 8.

5 The integer m in formula (II) is 0 to about 18. Preferably, m is about 2 to about 14. More preferably, m is about 4 to about 10.

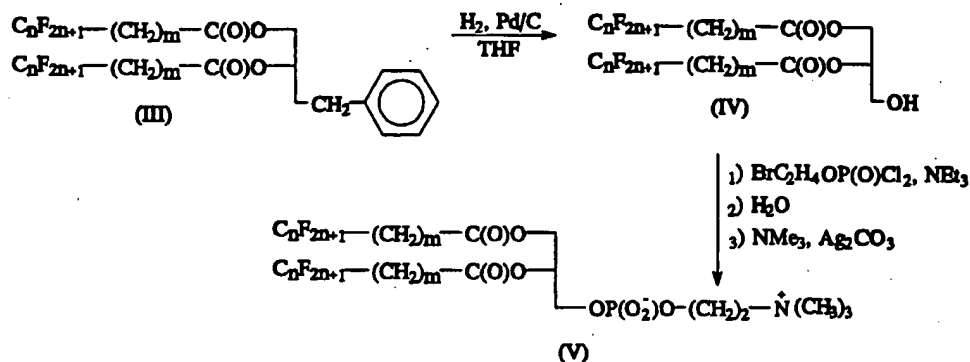
In formula (II) above, n is 1 to about 12. Preferably, n is about 2 to about 10, with about 4 to about 8 being more preferred.

10 The integer w in formula (II) is 1 to about 8. Preferably, w is 1 to about 4. More preferably, w is about 2.

The fluorinated amphiphilic compounds of the compositions of the present invention, including the compounds of formulas (I) and (II), can be prepared readily using standard organic synthetic methodology well known to those of ordinary skill in the art. Suitable methods for preparing fluorinated amphiphilic compounds are disclosed, for example, in C. Santaella et al., *New Journal of Chemistry*, 15, 685
 15 (1991), the disclosures of which are hereby incorporated by reference, in their entirety. Exemplary of the available methods for preparing fluorinated amphiphilic compounds are synthetic methods based on the phosphorylation of 1,2-di-(F-alkylacyl)-3-glycerol derivatives. These methods can be utilized in the preparation of
 20 perfluoroalkyl phosphatidylcholines, and particularly perfluoroalkyl phosphatidylethanolamines, and are disclosed, for example, in the aforementioned Santaella publication. Such methods involve linear phosphorylating agents, including the Hirt and Berchtold reagent (2-bromoethyldichlorophosphate (BEDP)), which is readily available in large quantities. Hirt et al., *Pharm. Acta. Helv.*, 33, 349 (1958).

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BEDP is highly reactive and can be used to phosphorylate sterically hindered disubstituted glycerols. Hansen et al., *Lipids*, 17, 453 (1982). The ammonium group can be introduced by reaction of an appropriate amine with the 1,2-diacylglycero-3-(2-bromoethyl)phosphate intermediate to give the desired phosphatidylethanolamine derivatives. This synthetic methodology is depicted in the following reaction scheme.



where m and n are as previously defined.

The 1,2-disubstituted-3-benzylglycerol derivatives of formula (III) are readily synthesized in high yields (85 to 90%) by acylation of 1-benzylglycerol with the corresponding perfluoroalkanoyl halides. *Proc. Natl. Acad. Sci. USA*, 75, 4074 (1978). The benzyl protecting group can be removed by hydrogenolysis over a palladium on charcoal catalyst (Pd/C) in tetrahydrofuran (THF). *Proc. Natl. Acad. Sci. USA*, 75, 4074 (1978). Short reaction times for the hydrogenolysis of the benzyl group are preferred to avoid transesterification of the 1,2-diacylglycerol of formula (III) into the more thermodynamically stable 1,3-diacylglycerol isomer. The hydrogenolysis reaction can be monitored by ordinary analytical techniques, including, for example, thin layer chromatography (TLC) and proton (^1H) nuclear magnetic resonance (NMR). The reaction is generally complete in about one hour with little or no transesterification. The hydrogenolysis reaction is preferably conducted in THF because both the starting material in the involved reaction (the compound of formula (III)) and the product (the compound of formula (IV)) tend to be highly soluble in THF. In addition, THF is conveniently used as the solvent in the subsequent phosphorylation step.

After hydrogenolysis, the catalyst (Pd/C) can be removed by filtration. The 1,2-diacylglycerols of formula (IV) can be reacted immediately with BEDP and

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an excess of triethylamine. Phosphorylation is typically completed in about 2 to about 4 hours, as measured by TLC. The remaining phosphochloride bond can be hydrolysed in aqueous base and generally requires about 22 hours for completion. When mineral bases or salts are used for hydrolysis including, for example, Na₂CO₃, KCl and EDTA sodium salt, the phosphate salts are highly insoluble in water or organic solvents. Fleischer, *Methods Enzymol.* 98, 263 (1983). The brominated intermediate can be isolated as a stable and soluble hydrogenotriethylammonium salt if excess triethylamine is used. Acidification of the phosphate salts to form the corresponding acid is difficult because the glycerol ester bonds are hydrolyzed at the necessary pH (pH of 2 to 3). Product degradation occurs also during purification over silica gel. Accordingly, it is preferred to use the phosphate salt without further purification. Nucleophilic displacement of the bromide ion by a large excess of trimethylamine occurs in a solvent mixture of CHCl₃/CH₃CN at 45°C over a 12 hour period. The displaced bromide ion can be precipitated by the addition of silver carbonate.

The concentration of fluorinated amphiphilic compound in the present compositions can vary and depends upon various factors, including, for example, the particular amphiphilic compound(s) which are employed in the compositions. Toxicity is generally of limited concern since the present fluorinated amphiphilic compounds are substantially biocompatible. See C. Santaella et al., *New Journal of Chemistry*, 15, 685 (1991). In general, the concentration of fluorinated amphiphilic compound in the present compositions is from about 0.001 mg/mL to about 200 mg/mL, with a concentration of about 0.01 mg/mL to 20 mg/mL being preferred. More preferably, the concentration of fluorinated amphiphilic compound is about 0.05 mg/mL to about 10 mg/mL, with a concentration of about 0.1 mg/mL to about 5 mg/mL being even more preferred.

The stabilized compositions of the present invention also comprise a gas, and preferably, an inert gas. The gases provide the compositions with enhanced reflectivity, particularly in vesicular composition in which the gas is entrapped within the vesicles. This increases their effectiveness as contrast agents.

Preferred gases are gases which are inert and which are biocompatible. Preferable gases include those selected from the group consisting of air, noble gases,

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such as helium, neon, argon and xenon, carbon dioxide, nitrogen, fluorine, oxygen, sulfur hexafluoride, fluorocarbons, perfluorocarbons, and mixtures thereof.

Perfluorocarbons are preferred gases. Preferably, the perfluorocarbon is selected from the group consisting of perfluoromethane, perfluoroethane, perfluoropropane,

- 5 perfluorobutane, perfluorocyclobutane and mixtures thereof. More preferably, the perfluorocarbon gas is selected from the group consisting of perfluoropropane and perfluorobutane, with perfluoropropane being particularly preferred. Another preferable gas is sulfur tetrafluoride.

- It is contemplated that mixtures of different types of gases, including
10 mixtures of a perfluorocarbon gas and another type of gas, such as air, can also be used in the compositions of the present invention. The gases discussed in Quay, International Application WO 93/05819, including the high "Q" factor gases described therein, may be used also. The disclosures of Quay, International Application WO 93/05819 are incorporated herein by reference in their entirety. In addition,
15 paramagnetic gases and gases of isotopes, such as ^{17}O , may be used. Other gases, including the gases exemplified above, would be readily apparent to one skilled in the art based on the present disclosure. The gases can be selected, as desired, to provide compositions which are suitable for use as contrast agents in ultrasound, as well as other diagnostic techniques, such as computed tomography (CT) and magnetic
20 resonance imaging (MRI).

- In certain preferred embodiments, a gas, for example, air or a perfluorocarbon gas, is combined with a liquid perfluorocarbon, such as perfluorohexane, perfluoroheptane, perfluorooctylbromide (PFOB), perfluorodecalin, perfluorododecalin, perfluorooctyliodide, perfluorotripropylamine and
25 perfluorotributylamine.

It may also be desirable to incorporate in the stabilized compositions a precursor to a gaseous substance. Such precursors include materials that are capable of being converted to a gas *in vivo*. Preferably, the gaseous precursor is biocompatible, and the gas produced *in vivo* is biocompatible also.

- 30 A variety of gaseous precursors are available for use in the present compositions including, for example, substances which are sensitive to changes to pH. These substances include materials that are capable of evolving gas, for example,

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upon exposure to a pH that is substantially neutral or acidic. Examples of such pH sensitive substances include salts of an acid selected from the group consisting of inorganic acids, organic acids and mixtures thereof. Carbonic acid (H_2CO_3) is an example of a suitable inorganic acid, and aminomalononic acid is an example of a
5 suitable organic acid. Other acids, including inorganic and organic acids, would be readily apparent to one skilled in the art based on the present disclosure.

Preferably, the gaseous precursor is a salt which is selected from the group consisting of an alkali metal salt, an ammonium salt and mixtures thereof. More preferably, the salt is selected from the group consisting of carbonate,
10 bicarbonate, sesquicarbonate, aminomalonate and mixtures thereof.

Exemplary of gaseous precursor materials for use in the compositions of the present invention and which are salts include lithium carbonate, sodium carbonate, potassium carbonate, lithium bicarbonate, sodium bicarbonate, potassium bicarbonate, magnesium carbonate, calcium carbonate, magnesium bicarbonate,
15 ammonium carbonate, ammonium bicarbonate, ammonium sesquicarbonate, sodium sesquicarbonate, sodium aminomalonate and ammonium aminomalonate.

Aminomalonate is well known in the art, and its preparation is described, for example, in Thanassi, *Biochemistry*, Vol. 9, no. 3, pp. 525-532 (1970); Fitzpatrick et al., *Inorganic Chemistry*, Vol. 13, no. 3 pp. 568-574 (1974); and Stelmashok et al.,
20 *Koordinatsionnaya Khimiya*, Vol. 3, no. 4, pp. 524-527 (1977). The disclosures of these publications are hereby incorporated herein by reference.

In addition to, or instead of, being sensitive to changes in pH, the gaseous precursor materials may also comprise compounds which are sensitive to changes in temperature. Such temperature sensitive agents include, for example,
25 materials which have a boiling point of greater than about 37°C at atmospheric pressure. For example, perfluoropentane has a liquid/gas phase transition temperature (boiling point) of 29.5°C at atmospheric pressure. This means that perfluoropentane will be a liquid at room temperature (about 25°C), but will become a gas within the human body, the normal temperature of which is 37°C, which is above the transition
30 temperature of perfluoropentane. Thus, under normal circumstances, perfluoropentane is a gaseous precursor. As a further example, there are the homologs of perfluoropentane, namely perfluorobutane and perfluorohexane. The liquid/gas

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transition of perfluorobutane is 4°C and that of perfluorohexane is 57°C. Thus, perfluorobutane is potentially useful as a gaseous precursor, although more likely as a gas, whereas perfluorohexane would likely be useful as a gaseous precursor because of its relatively high boiling point.

- 5 As discussed below, compositions containing gaseous precursors, such as perfluoropentane and/or perfluorohexane, can be prepared, for example, by agitating in a suitable container, such as a vial, an aqueous mixture of a fluorinated amphiphilic compound and a gaseous precursor at standard temperature and pressure. Under these conditions, the gaseous precursor would be in the form of a liquid.
- 10 Alternatively, compositions containing gaseous precursors can be prepared at elevated temperature and atmospheric pressure in which case the gaseous precursor can be volatilized. Thus, for example, when a composition of a fluorinated amphiphile and perfluoropentane is prepared, for example, at a temperature of about 35°C to about 40°C, the perfluoropentane is gaseous. The perfluoropentane would occupy the head
- 15 space of the container in which the composition is prepared.

 In addition to elevated temperature, amphiphilic compositions containing gaseous precursors can be prepared at about room temperature but at a reduced pressure. As known to those of ordinary skill in the art, the boiling point of various of the gaseous precursors described herein, including the perfluorocarbons,

20 such as perfluoropentane and perfluorohexane, is lowered to about room temperature or less under reduced pressure. Thus, gaseous precursors can be incorporated into the present amphiphilic compositions as a gas by preparing the compositions at a reduced pressure. The volatilized gaseous precursor would then occupy the head space of the container in which the composition is prepared.

- 25 A wide variety of materials which are sensitive to changes in temperature can be used as gaseous precursors in the compositions of the present invention. Suitable gaseous precursors include, for example, hexafluoroacetone, isopropyl acetylene, allene, tetrafluoroallene, boron trifluoride, 1,2-butadiene, 2,3-butadiene, 1,3-butadiene, 1,2,3-trichloro-2-fluoro-1,3-butadiene, 2-methyl-1,3-
- 30 butadiene, hexafluoro-1,3-butadiene, butadiyne, 1-fluorobutane, 2-methylbutane, perfluorobutane, 1-butene, 2-butene, 2-methyl-1-butene, 3-methyl-1-butene, perfluoro-1-butene, perfluoro-2-butene, 4-phenyl-3-butene-2-one, 2-methyl-1-butene-3-yne,

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- butyl nitrate, 1-butyne, 2-butyne, 2-chloro-1,1,1,4,4,4-hexafluorobutyne, 3-methyl-1-butyne, perfluoro-2-butyne, 2-bromo-butyraldehyde, carbonyl sulfide, crotononitrile, cyclobutane, methyl-cyclobutane, octafluorocyclobutane, perfluorocyclobutene, 3-chlorocyclopentene, perfluorocyclopentane, octafluorocyclopentene, cyclopropane,
- 5 perfluorocyclopropane, 1,2-dimethyl-cyclopropane, 1,1-dimethylcyclopropane, 1,2-dimethylcyclopropane, ethylcyclopropane, methylcyclopropane, diacetylene, 3-ethyl-3-methyl diaziridine, 1,1,1-trifluorodiazaoethane, dimethyl amine, hexafluorodimethylamine, dimethylethylamine, bis-(dimethylphosphine)amine, perfluorohexane, perfluoroheptane, perfluorooctane, 2,3-dimethyl-2-norbornane, perfluorodimethyl-
- 10 amine, dimethyloxonium chloride, 1,3-dioxolane-2-one, 4-methyl-1,1,1,2-tetrafluoroethane, 1,1,1-trifluoroethane, 1,1,2,2-tetrafluoroethane, 1,1,2-trichloro-1,2,2-trifluoroethane, 1,1-dichloroethane, 1,1-dichloro-1,2,2,2-tetrafluoroethane, 1,2-difluoroethane, 1-chloro-1,1,2,2,2-pentafluoroethane, 2-chloro-1,1-difluoroethane, 1,1-dichloro-2-fluoroethane, 1-chloro-1,1,2,2-tetrafluoroethane, 2-chloro-1,1-
- 15 difluoroethane, chloroethane, chloropentafluoroethane, dichlorotrifluoroethane, fluoroethane, perfluoroethane, nitropentafluoroethane, nitrosopentafluoroethane, perfluoroethylamine, ethyl vinyl ether, 1,1-dichloroethane, 1,1-dichloro-1,2-difluoroethane, 1,2-difluoroethane, methane, trifluoromethanesulfonylchloride, trifluoromethanesulfonylfluoride, bromodifluoronitrosomethane, bromofluoromethane,
- 20 bromochlorofluoromethane, bromotrifluoromethane, chlorodifluoronitromethane, chlorodinitromethane, chlorofluoromethane, chlorotrifluoromethane, chlorodifluoromethane, dibromodifluoromethane, dichlorodifluoromethane, dichlorofluoromethane, difluoromethane, difluoriodomethane, disilanomethane, fluoromethane, iodomethane, iodotrifluoromethane, nitrotrifluoromethane, nitrosotrifluoromethane, tetrafluoro-
- 25 methane, trichlorofluoromethane, trifluoromethane, 2-methylbutane, methyl ether, methyl isopropyl ether, methyl lactate, methylnitrite, methylsulfide, methyl vinyl ether, neopentane, nitrous oxide, 1,2,3-nonadecane-tricarboxylic acid-2-hydroxytrimethyl-ester, 1-nonene-3-yne, 1,4-pentadiene, *n*-pentane, perfluoropentane, 4-amino-4-methylpentan-2-one, 1-pentene, 2-pentene (cis), 2-pentene (trans), 3-bromopent-1-ene,
- 30 perfluoropent-1-ene, tetrachlorophthalic acid, 2,3,6-trimethylpiperidine, propane, 1,1,1,2,2,3-hexafluoropropane, 1,2-epoxypropane, 2,2-difluoropropane, 2-amino-

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propane, 2-chloropropane, heptafluoro-1-nitropropane, heptafluoro-1-nitrosopropane, perfluoropropane, propene, hexafluoropropane, 1,1,1,2,3,3-hexafluoro-2,3-dichloropropane, 1-chloropropane, chloropropane-(trans), 2-chloropropane, 3-fluoropropane, propyne, 3,3,3-trifluoropropyne, 3-fluorostyrene, sulfur (di)-decafluoride (S_2F_{10}),
5 2,4-diaminotoluene, trifluoroacetonitrile, trifluoromethyl peroxide, trifluoromethyl sulfide, tungsten hexafluoride, vinyl acetylene and vinyl ether. Examples of gaseous precursors are generally described, for example, in U.S. Patent Nos. 5,088,499 and 5,149,319, the disclosures of which are hereby incorporated herein by reference in their entirety.

10 Perfluorocarbons are preferred gaseous precursors for use in the compositions of the present invention. Exemplary of perfluorocarbon gaseous precursors are perfluorocarbons selected from the group consisting of perfluoropentane, perfluorohexane, perfluoroheptane, perfluorooctane and perfluorononane. Preferably, the perfluorocarbon gaseous precursor is selected from
15 the group consisting of perfluoropentane, perfluorohexane and perfluorooctane, with perfluoropentane being particularly preferred.

The gaseous precursor materials may be also photoactivated materials, such as diazonium ion and aminomalonate. As discussed more fully hereinafter, certain stabilized compositions, and particularly vesicular compositions, may be
20 formulated so that gas is formed at the target tissue or by the action of sound on the particle. Other gaseous precursors, in addition to those exemplified above, will be apparent to one skilled in the art based on the present disclosure.

As noted above, certain preferred embodiments of the present invention involve vesicular compositions. The size of the vesicles can be adjusted, if desired,
25 by a variety of procedures including, for example, microemulsification, vortexing, extrusion, filtration, sonication, homogenization, repeated cycles of freezing and thawing, extrusion under pressure through pores of defined size, and similar methods.

For intravascular use, the vesicles preferably have diameters of less than about 30 μm , and more preferably, less than about 12 μm . For targeted
30 intravascular use including, for example, binding to certain tissue, such as cancerous tissue, the vesicles can be significantly smaller, for example, less than 100 nm in diameter. For enteric or gastrointestinal use, the vesicles can be significantly larger,

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for example, up to a millimeter in size. Preferably, the vesicles are sized to have diameters between about 20 μm and 100 μm .

Tabulated below is a listing of a series of gaseous precursors which undergo phase transitions from liquid to gas at relatively close to normal human body temperature (37°C) or below. Also listed in the table are the sizes, in diameter, of emulsified droplets that would be required to form a vesicle of a maximum size of about 10 μm .

TABLE I

**Physical Characteristics of Gaseous Precursors and
Diameter of Emulsified Droplet to Form a 10 μm Vesicle**

Compound	Molecular Weight	Boiling Point (°C)	Density	Diameter (μm) of emulsified droplet to make 10 micron vesicle
perfluoropentane	288.04	29.5	1.7326	2.9
1-fluorobutane	76.11	32.5	6.7789	1.2
2-methylbutane (isopentane)	72.15	27.8	0.6201	2.6
2-methyl-1-butene	70.13	31.2	0.6504	2.5
2-methyl-2-butene	70.13	38.6	0.6623	2.5
1-butene-3-yne-2-methyl	66.10	34.0	0.6801	2.4
3-methyl-1-butyne	68.12	29.5	0.6660	2.5
octafluorocyclobutane	200.04	-5.8	1.48	2.8
decafluorobutane	238.04	-2	1.517	3.0
hexafluoroethane	138.01	-78.1	1.607	2.7

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Source: Chemical Rubber Company Handbook of Chemistry and Physics Robert C. Weast and David R. Lide, eds. CRC Press, Inc. Boca Raton, Florida. (1989 - 1990).

The utility of the compositions of the present invention can be optimized, for example, by using gases of limited solubility. Limited solubility, as
5 used herein, refers to the ability of the gas to diffuse, for example, out of vesicles by virtue of its solubility in the surrounding aqueous medium. A greater solubility in the aqueous medium imposes a gradient with the gas in the vesicle such that the gas will have a tendency to diffuse out of the vesicle. A lesser solubility in the aqueous medium will decrease the gradient between the vesicle and the interface such that the
10 diffusion of the gas out of the vesicle will be impeded. Preferably, the gas entrapped in the vesicle has a solubility less than that of oxygen, namely, 1 part gas in 32 parts water. See *Matheson Gas Data Book*, Matheson Company, Inc. (1966). More preferably, the gas entrapped in the vesicle possesses a solubility in water less than that of air; and even more preferably, the gas entrapped in the vesicle possesses a solubility
15 in water less than that of nitrogen.

The gases and/or gaseous precursors are preferably incorporated in the stabilized compositions of the present invention irrespective of the physical nature of the composition. Thus, it is contemplated that the gases and/or precursors thereto are incorporated in compositions in which the fluorinated amphiphilic compounds are
20 aggregated, for example, substantially randomly, as well as compositions in which the fluorinated amphiphilic compounds form vesicles, including micelles and liposomes. Incorporation of the gases and/or gaseous precursors in the present compositions may be achieved by using any of a number of methods. For example, the formation of gas-filled vesicles can be achieved by shaking or otherwise agitating an aqueous mixture
25 which comprises a gas or gaseous precursor and the fluorinated amphiphilic compounds. This promotes the formation of stabilized vesicles within which the gas or gaseous precursor is encapsulated.

In addition, a gas may be bubbled directly into an aqueous mixture of the fluorinated amphiphilic compounds. Alternatively, a gas instillation method can be
30 used as disclosed, for example, in U.S. Patent Nos. 5,352,435 and 5,228,446, the disclosures of each of which are hereby incorporated herein by reference in their entirety. Suitable methods for incorporating the gas or gaseous precursor in the

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present compositions are disclosed also in U.S. Patent No. 4,865,836, the disclosures of which are hereby incorporated herein by reference. Other methods would be apparent to one skilled in the art based on the present disclosure.

In preferred embodiments, the gases and/or gaseous precursor materials
5 are incorporated in vesicular compositions, with micelles and liposomes being preferred. As discussed in detail below, vesicles in which a gas or gas precursor or both are encapsulated are advantageous in that they provide improved reflectivity *in vivo*.

In certain embodiments, the stabilized compositions of the present
10 invention can further comprise one or more additional amphiphilic materials. Due to the amphipathy of these additional amphiphilic materials, they are generally capable, in preferred form, of enhancing or otherwise improving the stability of the present compositions. It has been found that incorporating one or more additional amphiphilic materials is particularly advantageous in connection with compositions involving
15 asymmetric fluorinated amphiphilic compounds. Particularly preferred additional amphiphilic materials include compounds which are biocompatible and which are capable of promoting the formation of vesicles, and especially stabilized vesicles, in the present stabilized compositions.

In certain preferred embodiments, the additional amphiphilic materials
20 comprise a lipid compound. Suitable lipids include, for example, phospholipids, such as phosphatidylcholine with both saturated and unsaturated fatty acids, including dioleoylphosphatidylcholine, dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine; phosphatidylethanolamines, such as dipalmitoylphosphatidylethanolamine, dioleoylphosphatidylethanolamine, N-succinyl-
25 dioleoylphosphatidylethanolamine and 1-hexadecyl-2-palmitoylglycerophosphoethanolamine; phosphatidylserine; phosphatidylglycerol; sphingolipids; glycolipids, such as ganglioside GM1; glucolipids; sulfatides; glycosphingolipids; phosphatidic acids, such as dipalmitoylphosphatidic acid; palmitic acid; stearic acid; arachidonic acid; oleic acid; lipids bearing polymers, such as polyethyleneglycol or polyvinylpyrrolidone;
30 cholesterol and cholesterol hemisuccinate; 12-(((7'-diethylaminocoumarin-3-yl)-carbonyl)methylamino)octadecanoic acid; N-[12-(((7'-diethylaminocoumarin-3-yl)-carbonyl)methylamino)octadecanoyl]-2-aminopalmitic acid; cholesteryl-(4'-trimethyl-

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amino)butanoate; 1,2-dioleoyl-sn-glycerol; 1,2-dipalmitoyl-sn-3-succinylglycerol; 1,3-dipalmitoyl-2-succinylglycerol; and palmitoylhomocysteine.

Suitable additional amphiphilic materials include also lipid compounds used to make mixed micelle systems, such as laurytrimethylammonium bromide; cetyl-
5 trimethylammonium bromide; myristyltrimethylammonium bromide; alkyltrimethyl-
benzylammonium chloride (where alkyl is, for example, C₁₂, C₁₄ or C₁₅);
benzyltrimethylundecylammonium bromide/chloride; benzyltrimethylhexadecyl-
ammonium bromide/chloride; benzyltrimethyltetradecylammonium bromide/chloride;
cetyltrimethylethylammonium bromide/chloride; and cetylpyridinium bromide/
10 chloride.

Suitable additional amphiphilic materials for use in the present compositions include also lipid compounds carrying a net charge, for example, anionic and/or cationic lipids. Exemplary cationic lipids include, for example, N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), dioleoyloxy-3-
15 (trimethylammonium)propane (DOTPA) and 1,2,-dioleoyloxy-e-(4'-trimethyl-
ammonium)butanoyl-sn-glycerol.

In addition to, or instead of, the lipid compounds described above, the additional amphiphilic materials may comprise aliphatic carboxylic acids, for example, fatty acids. Preferred fatty acids include those which contain about 5 to about 22
20 carbon atoms in the aliphatic group. The aliphatic group can be either linear or branched. Exemplary saturated fatty acids include, for example, (iso)lauric, (iso)myristic, (iso)palmitic and (iso)stearic acids. Exemplary unsaturated fatty acids include, for example, lauroleic, physeteric, myristoleic, palmitoleic, petroselinic, and oleic acid. Suitable fatty acids include also, for example, fatty acids in which the
25 aliphatic group is an isoprenoid or prenyl group.

The present compositions can also comprise, if desired, one or more neutral or positively or negatively charged materials. Exemplary neutral materials include, for example, oils, such as peanut oil, canola oil, olive oil, safflower oil and corn oil; lecithin; sphingomyelin; cholesterol and derivatives thereof; squalene;
30 terpenes and terpenoid compounds; triglycerides; gums, such as xanthan, tragacanth, locust bean, guar and carrageenan gums; methoxylated pectin; starch; agarose; cellulose and semi-synthetic cellulose, for example, methyl cellulose, hydroxyethyl

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cellulose, methoxy cellulose and hydroxypropyl cellulose; nonionic materials, including, for example, polyoxyethylene fatty acid esters, polyoxyethylene fatty alcohols, polyoxyethylene fatty alcohol ethers, polyoxyethylated sorbitan fatty acid esters, glycerol polyethylene glycol oxystearate, glycerol polyethylene glycol

5 ricinoleate, ethoxylated soybean sterols, ethoxylated castor oil, polyoxyethylene-polyoxypropylene polymers and polyoxyethylene fatty acid stearates; acacia; agar; bentonites, including purified bentonite; magma; carbomer 934P; dextrin; gelatin; di- and trihydroxy substituted alkanes and their polymers, including polyvinylalcohol; mono-, di- and triglycerides; amino alcohols; monosaccharides or sugar alcohols, such

10 as erythrose, threose, ribose, arabinose, xylose, lyxose, fructose, sorbitol, mannitol and sedoheptulose, with preferred monosaccharides being fructose, mannose, xylose, arabinose, mannitol and sorbitol; and disaccharides, such as lactose, sucrose, maltose and cellobiose.

Suitable positively charged materials include compounds containing, for

15 example, protonated or quaternary amino groups, including polymers in which the repeating units contain one or more amino groups, such as peptides, polypeptides, proteins and lipoproteins, such as albumin and natural lipoproteins. Exemplary positively charged materials include, for example, chitin; alkanolamines, such as monoethanolamine, diethanolamine and triethanolamine, and mixtures thereof,

20 including, for example, trolamine; polylysine; polyarginine; polyethyleneimine; chitosan; and peptides, including melanin concentrating hormone and dynorphin.

Suitable negatively charged materials are compounds containing, for example, carboxy (CO_2^-) groups, including polycarboxy polymers. Exemplary negatively charged materials include, for example, carboxymethylcellulose; salts of alginic acid, such as

25 sodium and calcium alginate; salts of glycosaminoglycans, including salts of hyaluronic acid; phosphorylated and sulfonated derivatives of carbohydrates; genetic material, such as interleukin-2 and interferon; phosphorothioate oligomers; and negatively charged peptides, such as deltorphin. In addition, carbohydrates bearing polymers may be used in the present compositions. Carbohydrate bearing lipids are described, for

30 example, in U.S. Patent No. 4,310,505, the disclosures of which are hereby incorporated by reference herein, in their entirety.

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In certain circumstances, it may be desirable to incorporate one or more charged species into the present amphiphilic compositions. It is believed that such charged species can contribute to the stability of the present amphiphilic compositions. Examples of suitable charged species include, for example, cations, such as metal ions, or anions. Exemplary cations include, for example, calcium, manganese, magnesium, copper, gadolinium or dysprosium cations, or any other cation which is compatible for use in connection with pharmaceutical applications. Suitable anions include, for example, sulphur, peroxides or superoxides. The anionic species may be chelated with chelating agents, for example, ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA).

Other materials which can be incorporated in the present compositions and which are contemplated as being capable of improving the stability of the compositions include polymeric materials. Exemplary of such polymeric materials include, for example, the polymers from which Z and/or R₆ in formula (I) are derived, as discussed hereinbefore.

It may be desirable to include in the amphiphilic compositions of the present invention anti-bactericidal agents and/or preservatives. Examples of these materials include, for example, sodium benzoate, quaternary ammonium salts, sodium azide, methyl paraben, propyl paraben, sorbic acid, ascorbyl, palmitate, butylated hydroxyanisole, butylated hydroxytoluene, chlorobutanol, dehydroacetic acid, ethylenediamine, monothioglycerol, potassium benzoate, potassium metabisulfite, potassium sorbate, sodium bisulfite, sulphur dioxide and organic mercurial salts.

Other additional materials which can be incorporated in the present compositions would be apparent to one skilled in the art based on the present disclosure. Preferably, the additional materials are selected to optimize certain desirable properties of the compositions, including serum stability and plasma half-life. This can be achieved without undue experimentation, based on the present disclosure.

The concentration of the additional materials in the present compositions can vary and depends, for example, upon the particular fluorinated amphiphilic compounds which are employed. In preferred embodiments, the concentration of additional materials is from about 0.01 mg/mL to about 200 mg/mL. More preferably, the concentration of additional materials is from about 0.05 mg/mL to about 5 mg/mL,

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with concentrations of about 15 mg/mL to about 2.5 mg/mL being even more preferred.

In certain preferred embodiments of the invention, the stabilized compositions comprise a vesicular composition. The vesicular compositions may
5 comprise micelles and/or liposomes. A wide variety of methods are available for the preparation of vesicular compositions, including, for example, shaking, drying, gas-installation, spray drying, and the like. Suitable methods for preparing the vesicular compositions are described, for example, in U.S. application Serial No. 307,305, filed September 16, 1994, the disclosures of which are incorporated herein by reference.

10 In preferred embodiments, fluorinated amphiphilic compounds are used in the preparation of the present compositions, including vesicular compositions, which are in the gel state at physiological temperature. As known to one of ordinary skill in the art, the main phase transition temperature (T_c) of nonfluorinated amphiphilic compounds, such as lipids, including, for example, phosphatidylcholines, is based
15 generally on the length of the carbon chains which are linked to the glycerol moiety. Exemplary representative lipids and their phase transition temperatures are set forth in the following table.

TABLE II

20 **Saturated Diacyl-sn-Glycero-3-Phosphocholines:
Main Chain Melting Transition Temperatures**

Number of Carbons in Acyl Chains	Main Phase Transition Temperature (°C)
1,2-(12:0)	-1.0
1,2-(13:0)	13.7
1,2-(14:0)	23.5
1,2-(15:0)	34.5
1,2-(16:0)	41.4

TABLE II**Saturated Diacyl-sn-Glycero-3-Phosphocholines:
Main Chain Melting Transition Temperatures**

Number of Carbons in Acyl Chains	Main Phase Transition Temperature (°C)
1,2-(17:0)	48.2
1,2-(18:0)	55.1
1,2-(19:0)	61.8
1,2-(20:0)	64.5
1,2-(21:0)	71.1
1,2-(22:0)	74.0
1,2-(23:0)	79.5
1,2-(24:0)	80.1

5

See, for example, Derek Marsh, *CRC Handbook of Lipid Bilayers*, p. 139 (CRC Press,
10 Boca Raton, FL 1990).

With respect to the fluorinated amphiphiles involved in the present
invention, it is believed that the number of carbon atoms which are fluorinated has a
greater effect on T_c , as opposed to the length of the chain(s), generally. In general,
the greater the number of carbons fluorinated in one or more of the chains, the higher
15 the T_c . Main chain transition temperatures of the compound of formula (V), which is
the product in the foregoing reaction scheme and which is depicted below, are set forth

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in the following table.

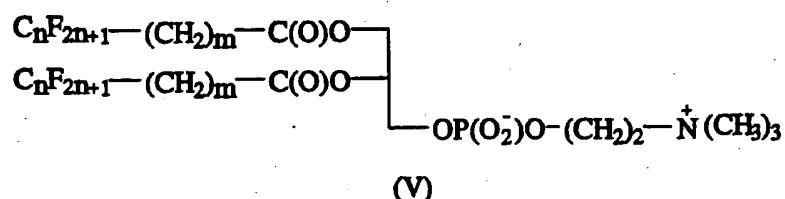


TABLE III

Perflourinated Diacyl-sn-Glycero-3-Phosphocholines:
Main Chain Melting Transition Temperatures

5	Compound (V)	m	n	Main Phase Transition Temperature (°C)
	(A)	4	4	a*
	(B)	4	6	a*
	(C)	4	8	69.3
	(D)	10	4	18.6
10	(E)	10	6	56.4
	(F)	10	8	> 95

An asterisk (*) means that no transition was detected between temperatures of 4°C and 95°C.

With particular reference to the preparation of micelle compositions, the following discussion is provided. Micelles may be prepared using any one of a variety of conventional micellar preparatory methods which will be apparent to those skilled in the art. These methods typically involve suspension of the fluorinated amphiphilic compound and additional amphiphilic compound, as desired, in an organic solvent, evaporation of the solvent, resuspension in an aqueous medium, sonication and centrifugation. The foregoing methods, as well as others, are discussed, for example,

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in Canfield et al., *Methods in Enzymology*, Vol. 189, pp. 418-422 (1990); El-Gorab et al., *Biochem. Biophys. Acta*, Vol. 306, pp. 58-66 (1973); *Colloidal Surfactant*, Shinoda, K., Nakagana, Tamamushi and Isejura, Academic Press, NY (1963) (especially "The Formation of Micelles", Shinoda, Chapter 1, pp. 1-88); *Catalysis in*
5 *Micellar and Macromolecular Systems*, Fendler and Fendler, Academic Press, NY (1975). The disclosures of each of the foregoing publications are incorporated by reference herein, in their entirety.

The vesicular composition may also comprise liposomes. In any given liposome, the involved amphiphilic compound(s), including fluorinated and
10 nonfluorinated amphiphilic compound, may be in the form of a monolayer or bilayer, and the mono- or bilayers may be used to form one or more mono- or bilayers. In the case of more than one mono- or bilayer, the mono- or bilayers are generally concentric. Thus, the amphiphilic compounds may be used to form unilamellar liposomes (comprised of one monolayer or bilayer), oligolamellar liposomes
15 (comprised of two or three monolayers or bilayers) or multilamellar liposomes (comprised of more than three monolayers or bilayers).

A wide variety of methods are available in connection with the preparation of liposome compositions. Accordingly, the liposomes may be prepared using any one of a variety of conventional liposomal preparatory techniques which will
20 be apparent to those skilled in the art. These techniques include solvent dialysis, French press, extrusion (with or without freeze-thaw), reverse phase evaporation, microemulsification and simple freeze-thawing. The liposomes may also be prepared by various processes which involve shaking or vortexing. This may be achieved, for example, by the use of a mechanical shaking device, such as a Wig-L-Bug™ (Crescent
25 Dental, Lyons, IL). Conventional microemulsification equipment, such as a Microfluidizer™ (Microfluidics, Woburn, MA) may be used also.

Additional methods for the preparation of liposome compositions include, for example, sonication, chelate dialysis, homogenization, solvent infusion, spontaneous formation, solvent vaporization, controlled detergent dialysis, and others,
30 each involving the preparation of liposomes in various fashions. Methods which involve freeze-thaw techniques are preferred in connection with the preparation of liposomes. Suitable freeze-thaw techniques are described, for example, in copending

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U.S. application Serial No. 07/838,504, filed February 19, 1992, the disclosures of which are incorporated herein by reference in their entirety. Preparation of the liposomes may be carried out in a solution, such as an aqueous saline solution, aqueous phosphate buffer solution, or sterile water.

5 As noted above, the size of the vesicles, including liposomes, can be adjusted, if desired, by a variety of techniques, including extrusion, filtration, sonication and homogenization. In addition, the size of the liposomes can be adjusted by the introduction of a laminar stream of a core of liquid into an immiscible sheath of liquid. Other methods for adjusting the size of the liposomes and for modulating the
10 resultant liposomal biodistribution and clearance of the liposomes would be apparent to one skilled in the art based on the present disclosure. Preferably, the size of the liposomes is adjusted by extrusion under pressure through pores of a defined size. Although liposomes employed in the subject invention may be of any one of a variety of sizes, the liposomes are preferably small, that is, less than about 100 nanometer
15 (nm) in outside diameter.

Many of the foregoing liposomal preparatory techniques, as well as others, are discussed, for example, in U.S. Patent No. 4,728,578; U.K. Patent Application GB 2193095 A; U.S. Patent No. 4,728,575; U.S. Patent No. 4,737,323; International Application Serial No. PCT/US85/01161; Mayer et al., *Biochimica et*
20 *Biophysica Acta*, Vol. 858, pp. 161-168 (1986); Hope et al., *Biochimica et Biophysica Acta*, Vol. 812, pp. 55-65 (1985); U.S. Patent No. 4,533,254; Mayhew et al., *Methods in Enzymology*, Vol. 149, pp. 64-77 (1987); Mayhew et al., *Biochimica et Biophysica Acta*, Vol. 755, pp. 169-74 (1984); Cheng et al., *Investigative Radiology*, Vol. 22, pp. 47-55 (1987); International Application Serial No. PCT/US89/05040;
25 U.S. Patent No. 4,162,282; U.S. Patent No. 4,310,505; U.S. Patent No. 4,921,706; and *Liposome Technology*, Gregoriadis, G., ed., Vol. I, pp. 29-31, 51-67 and 79-108 (CRC Press Inc., Boca Raton, FL 1984), the disclosures of each of which are hereby incorporated by reference herein, in their entirety.

Although any of a number of varying techniques can be used, it is
30 contemplated that the vesicular compositions of the present invention are preferably prepared using a shaking technique. Preferably, the shaking technique involves agitation with a mechanical shaking apparatus, such as a Wig-L-Bug™ (Crescent

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Dental, Lyons, IL), such as those disclosed in copending U.S. application Serial No. 160,232, filed November 30, 1993, the disclosures of which are hereby incorporated herein by reference in their entirety.

In certain embodiments of the present invention, the compositions further comprise a bioactive agent. These compositions are referred to herein as "amphiphilic formulations", and can be used for the therapeutic delivery *in vivo* of bioactive agents. Preferably, the amphiphilic formulations comprise vesicular formulations. In vesicular formulations, circulation and delivery of the vesicles to the targeted tissue can be observed via a non-invasive procedure. In connection with gas-filled or gaseous precursor-filled vesicles, the application of high energy ultrasound, radio frequency, optical energy, for example, laser light, and/or heat, to produce areas of hyperthermia, can be used, if desired, to rupture the vesicles *in vivo* and thereby promote release of the entrapped gas and/or gaseous precursor and bioactive agent. Thus, vesicular formulations permit the controlled release of a bioactive agent *in vivo*.

As those skilled in the art will recognize, any of the present stabilized compositions and/or formulations may be lyophilized for storage, and reconstituted, for example, with an aqueous medium (such as sterile water or phosphate buffered solution, or aqueous saline solution), with the aid of vigorous agitation. To prevent agglutination or fusion of the fluorinated amphiphilic compounds and/or additional amphiphilic compounds as a result of lyophilization, it may be useful to include additives which prevent such fusion or agglutination from occurring. Additives which may be useful include sorbitol, mannitol, sodium chloride, glucose, trehalose, polyvinylpyrrolidone and poly(ethylene glycol), for example, PEG 400. These and other additives are described in the literature, such as in the U.S. Pharmacopeia, USP XXII, NF XVII, The United States Pharmacopeia, The National Formulary, United States Pharmacopeial Convention Inc., 12601 Twinbrook Parkway, Rockville, MD 20852, the disclosures of which are hereby incorporated herein by reference in their entirety. Lyophilized preparations generally have the advantage of greater shelf life.

In the case of vesicular compositions, including micelles and liposomes, it is contemplated that the bioactive agent is preferably entrapped within a void of the vesicles. In certain cases, the bioactive agent can be incorporated also into the membrane walls of the vesicle. In the case of amphiphilic compositions in which the

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amphiphilic compounds are substantially aggregated randomly, or substantially not aggregated, it is contemplated that the bioactive agent is generally dispersed homogeneously throughout the composition.

The bioactive agent which is incorporated in the present compositions is preferably a substance which is capable of exerting a therapeutic biological effect *in vitro* and/or *in vivo*. Pharmaceuticals, drugs and genetic material are examples of suitable bioactive agents. Examples of genetic materials include, for example, genes carried on expression vectors, such as plasmids, phagemids, cosmids, yeast artificial chromosomes (YACs) and defective- or "helper" viruses; anti-sense and sense oligonucleotides; phosphorothioate oligodeoxynucleotides; antigene nucleic acids; and single and double stranded RNA and DNA, including DNA which encodes at least a portion of a gene, for example, DNA which encodes for human leukocyte antigen (HLA), dystrophin, cystic fibrosis transmembrane receptor (CFTR), interleukin-2 (IL-2), tumor necrosis factor (TNF) and granulocyte-macrophage colony stimulating factor (GM-CSF). The DNA can also encode certain proteins which may be used in the treatment of various types of pathologies or conditions, including those which are associated with the loss or deterioration of immune competence. Such pathologies or conditions involving immune competence include, for example, acquired immune deficiency syndrome (AIDS), cancer, chronic viral infections, and autoimmune disease.

Specifically, DNA may be selected which expresses adenosine deaminase (ADA) for the treatment of ADA deficiency; growth hormone for the treatment of growth deficiency or to aid in the healing of tissues; insulin for the treatment of diabetes; luteinizing hormone releasing hormone (LHRH) antagonist as a birth control agent; LHRH for the treatment of prostate or breast cancer; tumor necrosis factor and/or interleukin-2 for the treatment of advanced cancers; high-density lipoprotein (HDL) receptor for the treatment of liver disease; thymidine kinase for the treatment of ovarian cancer, brain tumors, or human immunodeficiency virus (HIV) infection; HLA-B7 for the treatment of malignant melanoma; IL-2 for the treatment of neuroblastoma, malignant melanoma or kidney cancer; interleukin-4 (IL-4) for the treatment of cancer; HIV *env* for the treatment of HIV infection; antisense *ras/p53* for the treatment of lung cancer; and Factor VIII for the treatment of Hemophilia B. Such

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therapies are described, for example, in *Science*, Vol. 258, pp. 744-746 (1992), the disclosure of which is incorporated herein by reference in its entirety.

In certain embodiments, it is desirable to incorporate in the amphiphilic compositions materials which comprise, for example, a polymer, such as an alginic acid polymer, that is covalently linked to a bioactive agent. An example of such a material is nalidixic acid alginate in which a polymer (alginic acid) is covalently linked to a bioactive material (nalidixic acid). Such materials are desirable in that it is believed they are capable of improving and/or enhancing the stability of the amphiphilic compositions, as well as providing a source of bioactive agent. It is contemplated also that after administration, the materials are hydrolyzed *in vivo*, to bioactive agent (nalidixic acid) and polymer (alginic acid).

As with the stabilized amphiphilic compositions, a wide variety of techniques also exist for the preparation of stabilized amphiphilic formulations. For example, the formulations may be prepared from a mixture of amphiphilic compounds, bioactive agent and gas or gaseous precursor. In this case, stabilized compositions are prepared as described above in the presence of a bioactive agent. Thus, for example, micelles and liposomes can be prepared in the presence of a bioactive agent. The preparation can involve, for example, bubbling a gas directly into an aqueous mixture of the fluorinated amphiphilic compounds and additional amphiphilic materials, as desired, and bioactive agent. Alternatively, the amphiphilic compositions may be preformed from fluorinated amphiphilic compounds and gas or gaseous precursor. In the latter case, the bioactive agent is then added to the amphiphilic composition prior to use. For example, an aqueous mixture of liposomes and gas may be prepared to which the bioactive agent is added and which is agitated to provide the liposome formulation. The liposome formulation is readily isolated also in that the gas- and/or bioactive agent-filled liposome vesicles generally float to the top of the aqueous solution. Excess bioactive agent can be recovered from the remaining aqueous solution.

The present amphiphilic compositions and/or formulations are suitable for diagnostic and/or therapeutic applications. In the case of diagnostic applications, such as ultrasound, the amphiphilic compositions, which may further comprise a gaseous precursor, are administered to a patient. Energy, preferably in the form of ultrasonic energy, is applied to at least a portion of the patient to image a region,

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which may include targeted tissue. A visible image of an internal region of the patient is then obtained, such that the presence or absence of diseased tissue can be ascertained.

Ultrasonic imaging techniques, including second harmonic imaging, are well known in the art, and are described, for example, in Uhlendorf, "Physics of Ultrasound Contrast Imaging: Scattering in the Linear Range", *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*, Vol. 14(1), pp. 70-79 (1994) and Sutherland, *et al.*, "Color Doppler Myocardial Imaging: A New Technique for the Assessment of Myocardial Function", *Journal of the American Society of Echocardiography*, Vol. 7(5), pp. 441-458 (1994), the disclosures of which are hereby incorporated herein by reference in their entirety.

With respect to therapeutic applications, the present amphiphilic formulations can be used in either *in vitro* or *in vivo* applications. In the case of *in vitro* applications, including cell culture applications, the amphiphilic formulations can be added to the cells in cultures and then incubated. If desired, where liposomes are employed, energy, such as sonic energy, may be applied to the culture media to burst the liposomes and release any therapeutic agents.

With respect to *in vivo* applications, the formulations of the present invention can be administered to a patient in a variety of forms adapted to the chosen route of administration, including, for example, parenteral, oral, or intraperitoneal. Parenteral administration, which is preferred, includes administration by the following routes: intravenous; intramuscular; interstitially; intra-arterially; subcutaneous; intraocular; intrasynovial; transepithelial, including transdermal; pulmonary via inhalation; ophthalmic; sublingual and buccal; topically, including ophthalmic; dermal; ocular; rectal; and nasal inhalation via insufflation. Intravenous administration is preferred among the routes of parenteral administration.

After vesicular formulations which comprise a gas and bioactive agent and, optionally, a gaseous precursor, have been administered to a patient, energy, preferably in the form of ultrasonic energy, can be applied to the target tissue to identify the location of the vesicles. The applied energy may also be employed to effect release of the bioactive agent and to facilitate cellular uptake of the bioactive agent. As one skilled in the art would recognize, based on the present disclosure, this

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method of mediating cellular uptake of bioactive agents with ultrasonic energy is preferably effected with tissues whose acoustic window permits the transmission of ultrasonic energy. This is the case for most tissues in the body, including, for example, muscle and organ tissues, such as the heart and liver, as well as most other
5 vital structures. With respect to brain tissue, it may be necessary to create a "surgical window" by removing part of the skull, inasmuch as ultrasonic energy generally does not transmit through bone. Intravascular and/or endoluminal ultrasound transducers may be used to apply the ultrasound energy to selected tissues and/or sites in the body, for example, the aorta and the esophagus.

10 Fluorinated amphiphilic formulations can be formulated to be sufficiently stable in the vasculature such that they circulate throughout the body and provide blood pool equilibration. As one skilled in the art would recognize, based on the present disclosure, the formulations, including those which comprise suspensions, emulsions and/or vesicles, such as liposomes and micelles, may be coated with certain materials
15 to minimize uptake by the reticuloendothelial system. Suitable coatings include, for example, gangliosides and glycolipids which bind saccharide moieties, such as glucuronate, galacturonate, guluronate, poly(ethylene glycol), poly(propylene glycol), polyvinylpyrrolidone, poly(vinyl alcohol), dextran, starch, phosphorylated and sulfonated mono-, di-, tri-, oligo- and polysaccharides and albumin. Provided that the
20 circulation half-life of the formulations is of a sufficient period of time, they will generally pass through the target tissue while passing through the body. In the case of formulations which comprise a bioactive agent, energy, for example, sonic energy, may be focused on the tissue to be treated, for example, diseased tissue. The bioactive agent will then be released locally in the target tissue. The inventors have found also
25 that antibodies, carbohydrates, peptides, glycopeptides, glycolipids and lectins also assist in the targeting of tissue. Accordingly, these materials may be incorporated into the fluorinated amphiphilic formulations also.

Different levels of energy are generally associated with diagnostic and therapeutic ultrasound. For example, the levels of energy associated with diagnostic
30 ultrasound are generally insufficient to cause rupture of vesicles and/or to facilitate release and cellular uptake of the bioactive agents. Moreover, diagnostic ultrasound involves the application of one or more pulses of sound. Pauses between pulses

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permits the reflected sonic signals to be received and analyzed. The limited number of pulses used in diagnostic ultrasound limits the effective energy which is delivered to the tissue that is being studied.

On the other hand, higher energy ultrasound, for example, ultrasound
5 which is generated by therapeutic ultrasound equipment, is generally capable of causing rupture of the vesicles. In general, therapeutic ultrasound machines use from about 10 to about 100% duty cycles, depending on the area of tissue to be treated with the ultrasound. Areas of the body which are generally characterized by larger amounts of muscle mass, for example, backs and thighs, as well as highly vascularized tissues,
10 such as heart tissue, may require a larger duty cycle, for example, up to about 100%.

In therapeutic ultrasound, continuous wave ultrasound is used to deliver higher energy levels. For the rupture of vesicles, continuous wave ultrasound is preferred, although the sound energy may be pulsed also. If pulsed sound energy is used, the sound will generally be pulsed in echo train lengths of about 8 to about 20 or
15 more pulses at a time. Preferably, the echo train lengths are about 20 pulses at a time. In addition, the frequency of the sound used may vary from about 0.25 to about 100 megahertz (MHz). In general, frequency for therapeutic ultrasound ranges between about 0.75 and about 3 MHz are preferred with about 1 and about 2 MHz being more preferred. In addition, energy levels may vary from about 0.5 Watt (W) per square
20 centimeter (cm^2) to about 5.0 W/cm^2 , with energy levels of about 0.5 to about 2.5 W/cm^2 being preferred. Energy levels for therapeutic ultrasound involving hyperthermia are generally from about 5 W/cm^2 to about 50 W/cm^2 . For very small vesicles, for example, vesicles having a diameter of less than about 0.5 micron, higher frequencies of sound are generally preferred. This is because smaller vesicles are
25 capable of absorbing sonic energy more effectively at higher frequencies of sound. When very high frequencies are used, for example, greater than about 10 MHz, the sonic energy will generally penetrate fluids and tissues to a limited depth only. Thus, external application of the sonic energy may be suitable for skin and other superficial tissues. However, for deep structures it is generally necessary to focus the ultrasonic
30 energy so that it is preferentially directed within a focal zone. Alternatively, the ultrasonic energy may be applied via interstitial probes, intravascular ultrasound catheters or endoluminal catheters. Such probes or catheters may be used, for

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example, in the esophagus for the diagnosis and/or treatment of esophageal carcinoma. In addition to the therapeutic uses discussed above, the present compositions can be employed in connection with esophageal carcinoma or in the coronary arteries for the treatment of atherosclerosis, as well as the therapeutic uses described, for example, in 5 U.S. Patent No. 5,149,319, the disclosures of which are hereby incorporated herein by reference in their entirety.

The useful dosage to be administered and the particular mode of administration will vary depending upon such factors as the age, weight and the particular animal and region thereof to be treated, the particular amphiphilic compound 10 or compounds used, the presence or absence of a bioactive agent, the diagnostic or therapeutic use contemplated, and the form of the involved compositions, for example, micelle or liposome, as will be readily apparent to those skilled in the art. Typically, dosage is administered at lower levels and increased until the desirable therapeutic effect is achieved. The amount of amphiphilic compound that is administered can vary 15 and generally depends upon the amount of particular fluorinated amphiphilic compound and additional amphiphilic material, as desired, administered.

The present invention is further described in the following examples. Examples 1, 2 and 4 to 7 describe the preparation of vesicular compositions within the scope of the present invention. Example 1 also describes an evaluation of the pressure 20 stability of a vesicular composition within the scope of the present invention and a composition prepared from a nonfluorinated amphiphilic compound. Example 3 describes the freeze-fracture analysis of vesicular compositions within the scope of the present invention. All of the examples are prophetic examples. These examples are for illustrative purposes only, and are not to be construed as limiting the appended 25 claims.

EXAMPLES

In the following examples, reference to the compounds of formulas (V)(A), (V)(E) and (V)(F) are to the corresponding compounds set forth in Table III above. In addition, "DPPC" in Example 1 refers to dipalmitoylphosphatidylcholine.

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Example 1

The compound of formula (V)(A) will be prepared using the procedures described hereinbefore. The compound will be suspended, at a concentration of 1 mg/mL, in a solution of saline:glycerol:propylene glycol (8:1:1 w/w/w). The resulting mixture (1.5 mL) will be placed into a sterile 3 mL glass vial. The head space in the vial will be replaced with perfluoropropane gas and the vial will be sealed. The vial will be shaken for 5 minutes at 4,200 r.p.m. on a Wig-L-Bug™ (Crescent Dental, Lyons, IL) shaking apparatus to provide perfluoropropane gas-filled vesicles (liposomes) having a mean diameter of about 3 μ m. The gas-filled vesicles will be stable for several weeks at room temperature. This will be repeated except that the compound of formula (V)(A) is replaced with DPPC. The pressure stability of the vesicular composition comprising the fluorinated amphiphile of formula (V)(A) and the composition containing DPPC will be compared in an acoustic laboratory. The compositions will be exposed to elevated pressure (300 mm Hg), and the loss in attenuation and backscatter between the compositions will be compared. The vesicular composition comprising the fluorinated amphiphilic compound of formula (V)(A) will exhibit improved pressure stability.

Example 2

The compound of formula (V)(F) will be prepared as described above. A vesicular composition comprising this compound will then be prepared using the procedure described in Example 1 except that the concentration of the saline:glycerol:propylene glycol suspension will be 1.25 mg/mL and the head space of the vial will be replaced with perfluorobutane gas. The resulting perfluorobutane gas-filled vesicular composition will exhibit improved properties, including pressure stability.

Example 3

Freeze-fracture electron microscopy (FFEM), using standard techniques, will be performed on the vesicular compositions within the scope of the present invention and which were prepared in Examples 1 and 2. FFEM will show that the

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gas-filled vesicles of the present invention possess smooth walls having a thickness of about a single bilayer.

Example 4

The compound of formula (V)(E) will be prepared using the procedures described above. A mixture of the compound of formula (V)(E) (10 mg/mL) in normal saline with trehalose (20 mg/mL) and poloxamer F68 (polyoxyethylene-polyoxypropylene glycol block copolymer) (25 mg/mL) will be heated to 60°C and passed through a Microfluidizer (Microfluidics Corp., Newton, MA) 20 times at 16,000 psi. The resulting vesicular composition will be lyophilized and the head space of the container in which the composition is stored will be instilled gradually with nitrogen gas and reequilibrated to ambient pressure over a period of 48 hours. The resulting vesicular composition will comprise gas (nitrogen) filled vesicles (liposomes) having a mean diameter of 200 nm. The vesicles can be stored dry until use, as desired, and can be rehydrated with sterile water and shaken prior to injection.

Example 5

The compound of formula (V)(E) will be prepared as described above and suspended, at a concentration of 1 mg/mL, in a solution of saline:glycerol:propylene glycol (8:1:1 w/w/w). The resulting mixture (1.5 mL) will be placed into a sterile 3 mL glass vial at 35°C. The head space in the vial will be replaced with perfluoropentane gas and the vial will be sealed. The vial will be shaken for 5 minutes at 4,200 r.p.m. on a Wig-L-Bug™ (Crescent Dental, Lyons, IL) shaking apparatus while the temperature of the vial is maintained at 35°C. Perfluoropentane gas-filled vesicles (liposomes) will be obtained.

Example 6

Example 5 will be repeated except that the perfluoropentane gas is replaced with a mixture of perfluoropentane gas and nitrogen gas (1:1 v/v). Cooling the resulting liposomes to below 30°C may result in the condensation of the perfluoropentane gas. However, the condensed perfluoropentane will exist as a nanodroplet within the vesicles and can be volatilized by warming to provide the gas-

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filled vesicles. This warming can be caused by artificial means *ex vivo* or by the body temperature of a patient after administration of the vesicular composition.

Example 7

The compound of formula (V)(F) will be prepared as described above
5 and suspended, at a concentration of 1 mg/mL, in a solution of
saline:glycerol:propylene glycol (7:1.5:1.5 w/w/w). The resulting mixture (1.5 mL)
will be placed into a sterile 3 mL glass vial at 60°C. The head space in the vial will
be replaced with perfluorohexane gas at 60°C. The vial will be sealed and shaken for
5 minutes at 4,400 r.p.m. on a Wig-L-Bug™ (Crescent Dental, Lyons, IL) shaking
10 apparatus while the temperature of the vial is maintained at 60°C. Perfluorohexane
gas-filled vesicles (liposomes) will be obtained.

The disclosures of each patent, patent application and publication cited
or described in this document are hereby incorporated by reference, in their entirety.

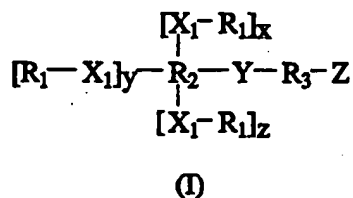
Various modifications of the invention, in addition to those described
15 herein, will be apparent to those skilled in the art from the foregoing description.
Such modifications are also intended to fall within the scope of the appended claims.

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What is claimed is:

CLAIMS

1. A stabilized composition comprising, in combination with a gas, a compound of the formula



5 wherein:

each of x, y and z is independently 0 or 1;

each X_1 is independently -O-, -S-, -SO-, -SO₂-, -NR₄-, -C(=X₂)-, -C(=X₂)-O-, -O-C(=X₂)-, -C(=X₂)-NR₄- or -NR₄-C(=X₂)-;

X_2 is O or S;

10 Y is a direct bond or -X₃-M(=O)(OR₅)_q-O-, where q is 1 or 2;

X_3 is a direct bond or -O-;

M is P or S;

Z is hydrogen, the residue of a hydrophilic polymer, a saccharide residue or -N(R₆)_r, where r is 2 or 3;

15 each R₁ is independently alkyl of 1 to about 30 carbons or fluorinated alkyl of 1 to about 30 carbons;

R₂ is a direct bond or an alkylene linking group of 1 to about 10 carbons;

20 R₃ is a direct bond or an alkylene diradical of 1 to about 10 carbons; and

each of R₄ and R₅ is independently hydrogen or alkyl of 1 to about 8 carbons; and

each R₆ is independently hydrogen, alkyl of 1 to about 8 carbons or a residue of a hydrophilic polymer;

25 provided that at least one of x, y and z is 1, at least one of R₁ is fluorinated alkyl of 1 to about 30 carbons, and when R₂ is a direct bond, two of x, y and z are 0.

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2. A composition according to Claim 1 wherein:
two of x, y and z are each 0.
3. A composition according to Claim 1 wherein:
one of x, y and z is 0 or 1; and
the other two of x, y and z are each 1.
4. A composition according to Claim 3 wherein:
one of x, y and z is 0; and
the other two of x, y and z are each 1.
5. A composition according to Claim 3 wherein:
each of x, y and z is 1.
6. A composition according to Claim 1 wherein:
 X_2 is O.
7. A composition according to Claim 6 wherein:
each X_1 is independently -O-, -S-, -C(=X₂)-, -C(=X₂)-O-,
-O-C(=X₂)-, -C(=X₂)-NR₄- or -NR₄-C(=X₂)-.
8. A composition according to Claim 7 wherein:
each X_1 is independently -C(=X₂)-O- or -O-C(=X₂)-.
9. A composition according to Claim 8 wherein:
 X_1 is -C(=X₂)-O-.
10. A composition according to Claim 9 wherein:
Y is -X₃-M(=O)(OR₃)₄.

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11. A composition according to Claim 10 wherein:
q is 1, X_3 is a direct bond, and M is P.
12. A composition according to Claim 11 wherein:
Z is $-N(R_6)_r$.
- 5 13. A composition according to Claim 3 wherein:
at least one of R_1 is alkyl of 1 to about 30 carbons and at least
one of R_1 is fluorinated alkyl of 1 to about 30 carbons.
- 10 14. A composition according to Claim 3 wherein:
each R_1 is independently fluorinated alkyl of 1 to about 30
carbons.
- 15 15. A composition according to Claim 3 wherein:
each R_1 is independently polyfluorinated alkyl of 1 to about 30
carbons.
- 16 16. A composition according to Claim 15 wherein:
each R_1 is independently perfluorinated alkyl of 1 to about 30
carbons.
- 17 17. A composition according to Claim 16 wherein:
 R_1 is $C_nF_{2n+1}-(CH_2)_m-$, where n is 1 to about 12 and m is 0 to
about 18.
- 20 18. A composition according to Claim 17 wherein:
n is about 2 to about 10 and m is about 2 to about 14.
19. A composition according to Claim 18 wherein:
n is about 4 to about 8 and m is about 4 to about 10.

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20. A composition according to Claim 1 wherein:
R₂ is a direct bond or an alkylene linking group of 1 to about 4
carbons;
R₃ is a direct bond or an alkylene diradical of 1 to about 4
5 carbons; and
each of R₄, R₅ and R₆ is independently hydrogen or alkyl of 1 to
about 4 carbons.
21. A composition according to Claim 20 wherein:
R₂ is an alkylene linking group of about 3 carbons;
10 R₃ is an alkylene diradical of about 2 carbons;
each of R₄ and R₅ is hydrogen; and
R₆ is hydrogen or methyl.
22. A composition according to Claim 21 wherein:
15 R₂ is -CH₂-CH-CH₂-;
|
R₃ is -CH₂CH₂-; and
R₆ is methyl.
23. A composition according to Claim 1 which is a stabilized
vesicular composition.
- 20 24. A composition according to Claim 23 wherein said vesicles are
selected from the group consisting of micelles and liposomes.
25. A composition according to Claim 23 wherein said vesicles
comprise gas-filled vesicles.

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26. A composition according to Claim 1 wherein said gas is selected from the group consisting of air, nitrogen, carbon dioxide, oxygen, fluorine, helium, argon, xenon and neon.
27. A composition according to Claim 1 wherein said gas comprises
5 a fluorinated gas.
28. A composition according to Claim 27 wherein said fluorinated gas is selected from the group consisting of a perfluorocarbon gas and sulfur hexafluoride.
29. A composition according to Claim 28 wherein said
10 perfluorocarbon gas is selected from the group consisting of perfluoromethane, perfluoroethane, perfluoropropane, perfluorobutane and perfluorocyclobutane.
-
30. A composition according to Claim 29 wherein said perfluorocarbon gas is selected from the group consisting of perfluoropropane and perfluorobutane.
31. A composition according to Claim 30 wherein said
15 perfluorocarbon gas comprises perfluoropropane.
32. A composition according to Claim 1 wherein said gas is derived, at least in part, from a gaseous precursor.

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33. A composition according to Claim 1 further comprising a gaseous precursor.

34. A composition according to Claim 33 wherein said gaseous precursor comprises a perfluorocarbon having a boiling point of greater than about
5 room temperature.

35. A composition according to Claim 34 wherein said gaseous precursor is selected from the group consisting of perfluoropentane, perfluorohexane and perfluorooctane.

36. A composition according to Claim 35 wherein said gaseous
10 precursor comprises perfluoropentane.

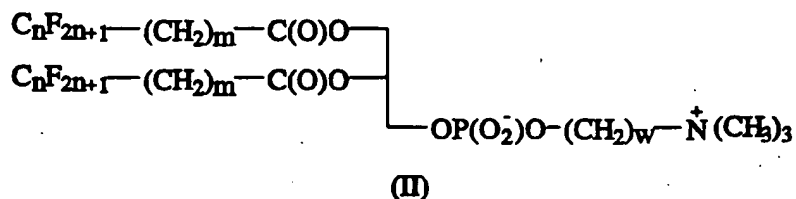
37. A composition according to Claim 1 further comprising a bioactive agent.

38. A composition according to Claim 1 which is for diagnostic imaging.

15 39. A composition according to Claim 38 wherein said imaging comprises ultrasound.

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40. A composition comprising, in combination with a gas, a compound of the formula



wherein:

m is 0 to about 18;

n is 1 to about 12; and

w is 1 to about 8.

41. A composition according to Claim 40 wherein

m is about 2 to about 14;

n is about 2 to about 10; and

w is 1 to about 4.

42. A composition according to Claim 41 wherein

m is about 4 to about 10;

n is about 4 to about 8; and

w is about 2.

43. A composition according to Claim 40 which is a stabilized vesicular composition.

44. A composition according to Claim 43 wherein said vesicles are selected from the group consisting of micelles and liposomes.

45. A composition according to Claim 43 wherein said vesicles comprise gas-filled vesicles.

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46. A composition according to Claim 40 wherein said gas is selected from the group consisting of air, nitrogen, carbon dioxide, oxygen, fluorine, helium, argon, xenon and neon.

47. A composition according to Claim 40 wherein said gas comprises
5 a fluorinated gas.

48. A composition according to Claim 47 wherein said fluorinated gas is selected from the group consisting of a perfluorocarbon gas and sulfur hexafluoride.

49. A composition according to Claim 48 wherein said
10 perfluorocarbon gas is selected from the group consisting of perfluoromethane, perfluoroethane, perfluoropropane, perfluorobutane and perfluorocyclobutane.

50. A composition according to Claim 49 wherein said perfluorocarbon gas is selected from the group consisting of perfluoropropane and perfluorobutane.

15 51. A composition according to Claim 50 wherein said perfluorocarbon gas comprises perfluoropropane.

52. A composition according to Claim 40 wherein said gas is derived, at least in part, from a gaseous precursor.

53. A composition according to Claim 40 further comprising a
20 gaseous precursor.

54. A composition according to Claim 53 wherein said gaseous precursor comprises a perfluorocarbon having a boiling point of greater than about room temperature.

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55. A composition according to Claim 54 wherein said gaseous precursor is selected from the group consisting of perfluoropentane, perfluorohexane and perfluorooctane.
56. A composition according to Claim 55 wherein said gaseous precursor comprises perfluoropentane.
57. A composition according to Claim 40 further comprising a bioactive agent.
58. A stabilized composition comprising a gas and a sulfonated or phosphorylated fluorinated amphiphilic compound.
59. A composition according to Claim 58 which is a stabilized vesicular composition.
60. A composition according to Claim 59 wherein said vesicles are selected from the group consisting of micelles and liposomes.
61. A composition according to Claim 59 wherein said vesicles comprise gas-filled vesicles.
62. A composition according to Claim 58 wherein said gas is selected from the group consisting of air, nitrogen, carbon dioxide, oxygen, fluorine, helium, argon, xenon and neon.
63. A composition according to Claim 58 wherein said gas comprises a fluorinated gas.
64. A composition according to Claim 63 wherein said fluorinated gas is selected from the group consisting of a perfluorocarbon gas and sulfur hexafluoride.

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65. A composition according to Claim 64 wherein said perfluorocarbon gas is selected from the group consisting of perfluoromethane, perfluoroethane, perfluoropropane, perfluorobutane and perfluorocyclobutane.

66. A composition according to Claim 65 wherein said gas is selected
5 from the group consisting of perfluoropropane and perfluorobutane.

67. A composition according to Claim 66 wherein said gas comprises perfluoropropane.

68. A composition according to Claim 58 wherein said fluorinated amphiphilic compound comprises a fluorinated lipid.

10 69. A composition according to Claim 68 wherein said fluorinated lipid comprises a fluorinated phospholipid.

70. A composition according to Claim 69 wherein an organic chain of said phospholipid is fluorinated.

71. A composition according to Claim 70 wherein said fluorinated
15 phospholipid comprises a fluorinated monochain phospholipid.

72. A composition according to Claim 70 wherein said fluorinated phospholipid comprises a fluorinated polychain phospholipid.

73. A composition according to Claim 72 wherein at least one of said chains is nonfluorinated.

20 74. A composition according to Claim 58 further comprising a gaseous precursor.

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75. A composition according to Claim 74 wherein said gaseous precursor comprises a perfluorocarbon having a boiling point of greater than about room temperature.

76. A composition according to Claim 75 wherein said gaseous precursor is selected from the group consisting of perfluoropentane, perfluorohexane and perfluorooctane.

77. A composition according to Claim 76 wherein said gaseous precursor comprises perfluoropentane.

78. A composition according to Claim 59 further comprising a bioactive agent.

79. A composition according to Claim 78 wherein said bioactive agent is substantially entrapped in said vesicles.

80. A stabilized composition consisting essentially of a gas and a fluorinated amphiphilic compound.

81. A stabilized composition consisting of a gas and a fluorinated amphiphilic compound.

82. A method for the preparation of a stabilized composition of a fluorinated amphiphilic compound and a gas comprising agitating an aqueous mixture of a fluorinated amphiphilic compound in the presence of a gas.

83. A method of providing an image of an internal region of a patient comprising (i) administering to the patient a contrast medium comprising a stabilized composition comprising a gas and a fluorinated amphiphilic compound, and (ii) scanning the patient using ultrasound to obtain visible images of the region.

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84. A method of providing an image of an internal region of a patient comprising (i) administering to the patient a vesicular composition comprising, in an aqueous carrier, vesicles comprising a fluorinated amphiphilic compound and a gas or gaseous precursor; and (ii) scanning the patient using ultrasound to obtain a visible
5 image of any diseased tissue in the patient.

85. A method for diagnosing the presence of diseased tissue in a patient comprising (i) administering to the patient a contrast medium comprising a stabilized composition comprising a gas and a fluorinated amphiphilic compound, and (ii) scanning the patient using ultrasound to obtain visible images of any diseased tissue
10 in the patient.

86. A method for the therapeutic delivery *in vivo* of a bioactive agent comprising administering to a patient a therapeutically effective amount of a formulation which comprises, in combination with a bioactive agent, a stabilized composition of a fluorinated amphiphilic compound and a gas.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/07813

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 49/00, 9/127; A61B 8/13; C07D 265/30

US CL :424/9.52; 544/84; 424/450

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/9.52, 9.5; 544/84; 424/450; 128/662.02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN REGISTRY, CAPLUS, MEDLINE, WPIDS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 5,344,930 A (RIESS ET AL.) 06 September 1994 (06.09.94), see entire document, especially abstract and column 1, lines 14-42.	1-81 ----- 82-86
Y	US 5,205,287 A (ERBEL ET AL.) 27 April 1993 (27.04.93), see entire document.	1-86
Y	US 4,900,540 A (RYAN ET AL.) 13 February 1990 (13.02.90), see columns 2-3.	82-86
X ----- Y	FREZARD et al. Fluorinated phospholipid-based vesicles as potential drug carriers: encapsulation/sustaining of drugs and stability in human serum. Artificial Cells and Blood Substitutes and Immobilization Biotechnology. 1994, Vol. 22, Issue 4, pages 1403-1408, see entire document.	1-81 ----- 82-86

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

02 AUGUST 1996

Date of mailing of the international search report

04 SEP 1996

 Name and mailing address of the ISA/US
 Commissioner of Patents and Trademarks
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/07813

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	FREZARD et al. Permeability and stability in buffer and in human serum of fluorinated phospholipid-based liposomes. Biochimica et Biophysica Acta. 1994, Vol. 1192, No. 1, pages 61-70, see entire document.	1-81 ----- 82-86

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/07813

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☒ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/07813

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-39, drawn to a stabilized composition comprising a gas, in combination with a gas, wherein the compound has a formula as shown in claim 1.

Group II, claim(s) 40-57, drawn to a composition comprising, in combination with a gas, a phosphate and fluorine containing compound having the formula as shown in claim 40.

Group III, claim(s) 58-79, drawn to a stabilized composition comprising a gas and a sulfonated or phosphorylated fluorinated amphiphilic compound.

Group IV, claim(s) 80-82, drawn to a stabilized composition consisting or consisting essentially of a gas and a fluorinated amphiphilic compound.

Group V, claim(s) 83-85, drawn to a method of providing imaging comprising administering to a patient a contrast medium comprising a stabilized composition comprising a gas and a fluorinated amphiphilic compound and then scanning the patient using ultrasound.

Group VI, claim(s) 86, drawn to a method of therapeutic delivery in vivo comprising a combination of a bioactive agent and a stabilized composition comprising a gas and a fluorinated amphiphilic compound.

The inventions listed as Groups I-VI do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical feature of Group I is a compound as shown by the formula in claim 1, which encompasses an almost infinite number of classes of organic compounds, while the special technical feature of Group II is a phosphate and fluorine containing organic compound having a more specific structure as shown by the formula in claim 40. The special technical feature of Group III is a sulfonated or phosphorylated amphiphilic compound, while the special technical feature of Group IV is a compound which is specified as a fluorinated amphiphilic compound. The special technical feature of Group V is a contrast agent which is administered for a method of generating an ultrasonic image, while the special technical feature of Group VI is a bioactive agent used in a method of in vivo therapeutic delivery. Since the special technical feature of any one of the Groups I-VI is not present in any of the other grouped inventions, unity of invention is lacking.